The study of molecular variation in Atlantic salmon (*Salmo salar* L.) and brown trout (*Salmo trutta* L.)

Ciar O'Toole, B.Sc. (Hons.), M.Sc.

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Research supervisors:
Professor Tom Cross, Dr. Philip McGinnity

Head of School: Professor John O'Halloran

School of Biological, Earth and Environmental Sciences
National University of Ireland, Cork

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Declaration

I declare that this thesis has not been previously submitted as an exercise for a degree at the National University of Ireland or any other university and I further declare that the work embodied in it is my own, or else noted.

Ciar O'Toole

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General Abstract

Polymorphic microsatellite DNA loci were used here in three studies, one on *Salmo salar* and two on *S. trutta*. In the case of *S. salar,* the survival of native fish and non-natives from a nearby catchment, and their hybrids, were compared in a freshwater common garden experiment and subsequently in ocean ranching, with parental assignment utilising microsatellites. Overall survival of non-natives was 35% of natives. This differential survival was mainly in the oceanic phase. These results imply a genetic basis and suggest local adaptation can occur in salmonids across relatively small geographic distances which may have important implications for the management of salmon populations. In the first case study with *S trutta,* the species was investigated throughout its spread as an invasive in Newfoundland, eastern Canada. Genetic investigation confirmed historical records that the majority of introductions were from a Scottish hatchery and provided a clear example of the structure of two expanding waves of spread along coasts, probably by natural straying of anadromous individuals, to the north and south of the point of human introduction. This study showed a clearer example of the genetic anatomy of an invasion than in previous studies with brown trout, and may have implications for the management of invasive species in general. Finally, the genetics of anadromous *S. trutta* from the Waterville catchment in south western Ireland were studied. Two significantly different population groupings, from tributaries in geographically distinct locations entering the largest lake in the catchment, were identified. These results were then used to assign very large rod caught sea trout individuals (so called "specimen" sea trout) back to region of origin, in a Genetic Stock Identification exercise. This suggested that the majority of these large sea trout originated from one of the two tributary groups. These results are relevant for the understanding of sea trout population dynamics and for the future management of this and other sea trout producing catchments. This thesis has demonstrated new insights into the population structuring of salmonids both between and within catchments. While these chapters look at the existence and scale of genetic variation from different angles, it might be concluded that the overarching message from this thesis should be to highlight the importance of maintaining genetic diversity in salmonid populations as vital for their long-term productivity and resilience.

Chapter 1:

General Introduction

This thesis uses molecular genetic methods, specifically microsatellite DNA, to investigate the issue of local adaptation in Atlantic salmon, *Salmo salar* (Linnaeus, 1798), then brown trout, *S. trutta* (Linnaeus, 1798), as an invasive species in eastern Canada, and finally to consider the population structure and assignment of large sexually mature rod caught fish to spawning tributary of *Salmo trutta* in a lacustrine location in south western (SW) Ireland.

1.1 Salmonid Biology and Life History

Trout and salmon are teleost fish of the order Salmoniformes, family Salmonidae, which contains about 68 species in nine genera. The more typical salmonids are the genus *Salmo* (Atlantic salmon and trout), *Oncorhynchus* (Pacific salmon and trout), *Salvelinus* (charrs) and *Hucho* (taimen and huchen) (Ade, 1989). Salmonids are native to the cool and cold waters in the Northern hemisphere. Their native range stretches from the Arctic drainages of Europe, Asia and Northern America as far south as the Mediterranean, northern Africa and northern Mexico where they spawn in fresh water, with anadromy occurring as a dominant life-history strategy in the northern part of the range (McDowall, 1988). Salmonid fishes have been widely introduced throughout the globe for various purposes over the past 150 years, mainly for sport fishing and aquaculture (Verspoor *et al.*, 2007) and have proven to be successful invasives in many areas (Quinn *et al.*, 2001, Ayllon *et al.*, 2004, Ayllon *et al.*, 2006, Launey, 2010, Valiente, *et al.*, 2010).

Salmonids all share the same basic life history requirements: they require cool, well oxygenated water in which to live and feed and are pollution sensitive. They reproduce in fresh water in shallow gravelly areas, and they are carnivorous and require suitably sized prey items at different stages of their life history (Ade, 1989). Salmonid fish differ from typical bony fishes in several ways. Their unusual traits include large eggs, maternal care, in terms of burial of eggs, anadromy and natal

homing. All salmonids spawn in freshwater and some spend all their lives there, but many salmonids migrate to sea to grow to their full adult size before returning to freshwater to spawn (Quinn, 2005). This life-history pattern leads to rapid growth and higher body mass than non-anadromous con-specifics. Anadromy is known to occur more frequently at higher latitudes, where growth opportunities in freshwater would be lower than those at sea, and so the cost and risk of migration is thought to be offset by the increased growth rate (McDowell, 1988). A particular feature of salmonid fishes is extremely accurate natal homing or philopatry (Quinn, 2005).

This thesis focuses on two species from the genus *Salmo*, *S. salar*, the Atlantic salmon, and *S. trutta*, the brown trout. Fish of the genus *Salmo* are well known for their diversity in terms of life history, ecology, morphology and behaviour, both between and within populations of the same species (Ferguson, 1989, Elliott, 1994, Fleming, 1998, Quinn, 2005).

Atlantic salmon *(Salmo salar*) spend the early stages of its life history in freshwater, before, in most cases, smoltifying and migrating to the marine environment to grow and mature sexually, before migrating back to their river of origin to spawn (natal homing). The historical distribution of Atlantic salmon is the North Atlantic and coastal drainages. Like most salmonids, Atlantic salmon show life-history variation within and between locations. Most common is the anadromous form, but landlocked and non-migratory populations occur, along with populations that only migrate to estuaries. Currently, Atlantic salmon populations in many historical areas are under threat or extinct, due to a combination of factors including overfishing, pollution, disease and habitat destruction, and overall throughout its native range Atlantic salmon is in decline (Verspoor *et al.*, 2007). Declines in recent years in marine returns of Atlantic salmon also indicate an as yet not fully understood mechanism affecting marine survival, possibly linked to climate change (Peyronnet *et al.*, 2008, Friedland *et al.*, 2014). Atlantic salmon are intensively farmed, being one of the world's main aquaculture species, with 99% of Atlantic salmon in existence at present being of farmed origin (Verspoor, 2007).

The majority of adult Atlantic salmon return from saltwater to the natal river/stream to spawn. Spawning occurs mainly between December-January in gravel beds

known as redds. The eggs develop into eyed ova (where eye and vertebrae are visible) before hatching in late February or March. This newly hatched fish is known as an alevin, and has an attached yolk sac. Once the yolk sac has been absorbed (after approx. 5-6 weeks), the fish is known as a fry. From when it leaves the redd until it begins to smoltify in readiness for life in the sea, it is known as a parr. In European waters, juvenile salmon can spend between one and six years in freshwater, dependant on water temperature, before it developing into a smolt. A smolt is a fully-silvered juvenile salmon migrating to sea to feed. The smolt run usually occurs in late spring to early summer. The returning adult salmon is either known as a grilse (one winter spent at sea) or, less commonly, a multi sea winter salmon (two or more winters at sea, MSW). These returning adults do not feed in freshwater, during the period prior to spawning. After spawning (between January and March), any surviving adult salmon will return to sea, spawned fish being referred to as kelts.

Variations to this archetypal life history pattern include mature male parr, non-anadromous populations and partially anadromous populations. Mature parr are male salmon, occurring within an anadromous population, that mature sexually at a small size before carrying out a marine migration, and typically exhibit "sneaking" mating tactics to avoid confrontation with the much larger adult mature males (Verspoor *et al.*, 2007). Non-anadromous populations are known to occur, both where there are barriers to a marine migration, and in some cases, where no such barrier occurs. There are also examples of Atlantic salmon populations that only migrate to the estuarine environment before returning to freshwater (Verspoor *et al.*, 2007).

The brown trout (*Salmo trutta),* is a salmonid species native to Europe, north west Asia and north Africa (Elliott, 1994). Brown trout exhibit two main life history strategies, either freshwater resident or anadromous. Freshwater resident fish spend their entire life cycle in freshwater, most regularly migrating to smaller streams or rivers to spawn, before returning to larger water bodies for feeding. Anadromous or migratory brown trout are those that spend a portion of their life history in the marine environment, and are often referred to as sea trout. This marine migration is related to feeding, although these fish return to freshwater to spawn (Jonsson and Jonsson, 1993, Crisp, 2000). Migratory sea trout differ in their colouration to

resident brown trout, being generally silver in appearance on their return to freshwater from the marine environment (Harris and Milner, 2006). Brown trout is a successful invasive in many regions worldwide but is also under threat in many parts of its native range, for reasons similar to those affecting Atlantic salmon populations (Ferguson, 2004). In addition to this, Irish sea trout populations were badly affected by sea lice, with poor marine returns occurring in several regions during the late 1980s. In many of these areas sea trout populations have yet to recover fully (Poole *et al.,* 2006).

The early stages of the brown trout life cycle are similar to Atlantic salmon, and while the species life history can vary considerably at later stages, there are some general similarities. Reproduction usually occurs by autumn spawning of mature fish in gravel beds in free flowing, clean, cool water. The eggs are buried in the gravel by the female, where they incubate over winter until hatching, and where the alevins then spend the first few weeks of life in the gravel until their yolk sacs have been absorbed (Jonsson and Jonsson, 2009). On emergence (in early spring in Ireland), juvenile brown trout begin external feeding and can spend several years in freshwater habitats that vary from small streams to lakes, depending on the habitat availability (Elliott, 1994).

Juveniles of both species (up to and including parr stage), prefer relatively shallow, fast-water areas, characterised by riffles and shallow pools. To allow full scope for activity in freshwater, both Atlantic salmon and brown trout require close to fully oxygen-saturated water (Gibson, 1993). Temperature is considered one of the most important variables relating to juvenile salmon and trout growth. At temperatures less than 9°C, young salmon seek shelter under coarse substrate or move to pools (Gardiner, 1984). Trout are generally considered more robust in relation to the extremes of temperature they can tolerate, allowing for more flexibility in normal behaviour and growth in freshwater (Elliott, 1994).

1.2 Molecular Techniques and Evolutionary Implications

Before the 1960s, and the development of various molecular methods for examining the genotypes of individual samples, population and individual genetic properties could only be inferred indirectly by descriptions of the phenotypes of organisms (Avise, 1994). The first studies to link ecology and molecular genetics used allozymes markers, where polymorphic protein variants, predominantly enzymes were visualised using gel electrophoresis and specific staining. This method reflected some of the variation present in DNA sequences, but was later criticized as insensitive for detecting variations in the underlying DNA (Schlotterer, 2005). Allozymes were followed by DNA based markers, such as in polymorphic mitochondrial DNA (mtDNA), and nuclear minisatellite loci. However, it was the development of the polymerase chain reaction (PCR) technique that allowed researchers to selectively amplify specific regions of DNA that could be isolated from small samples, from a variety of sources, e.g. blood, hair, soft tissue, fish scales (Freeland, 2005). DNA-based markers which utilised this technology included microsatellite loci, the marker used here. Some of the more recent developments involve the use of single nucleotide polymorphisms (SNPs) often detected by whole genome sequencing (Schlotterer, 2005).

The most commonly used markers for salmonid population genetics are currently mtDNA, microsatellites and SNPs. Mitochondrial DNA sequences are commonly used for examining evolutionary histories in animal species, and in phylogeographical studies (Avise, 1994). MtDNA is useful for this type of work due to its relatively high mutation rate and lack of recombination, but has some limitations in that it is maternally inherited, so describes only the pattern of spread of the maternal genotype and is essentially only representative of one locus (Hansen *et al.*, 2007). Microsatellite loci, the markers used throughout this study, are highly polymorphic and are appropriate for inferring recent events such as dispersal or mate choice. (Beebee and Rowe, 2004). Microsatellite loci are stretches of DNA that are made up of tandem repeats of 1-6bp. They are found throughout the nuclear genome and have also been found in the mitochondrial genomes of some species (Hartl, 2000). Microsatellite loci were traditionally sourced by cloning fragments of DNA

and using these fragments to create primers that amplify the repeat region (Freeland, 2005). Once primers have been designed, data can be generated by using the primers to amplify microsatellite loci in PCR reactions. These PCR products can then be run out on a high resolution gel or run through an automated laser based analysis system. The sequences that flank microsatellite loci frequently overlap between species, so microsatellite primers can often be used to generate data from multiple closely related species (Freeland, 2005). SNPs are the most recently developed of these three marker types. They are predominantly bi-allelic markers, consisting of single-base substitutions in nuclear DNA but their increased use in population genetics derives from the large numbers of SNPs that can be isolated, as these polymorphisms occur on average every 100-330 bp in a genome (Hansen *et al.,* 2007).

Molecular biology has had major impacts in species identification, animal behaviour, population genetics and conservation biology in the last fifty years (Beebee and Rowe, 2004). Natural populations can vary greatly in both size and structure, and this can have implications for their genetic make-up. Important measurable genetic features include total genetic diversity, extent of migration, differentiation and effective population size, which all help answer important ecological questions. These questions would otherwise either be unanswerable by traditional ecological methods, or require highly intensive, and often destructive, sampling methods (Allendorf and Luikart, 2007). Population genetic subdivision, for example, due to geographic barriers, can be measured using molecular markers and analysed using F-statistics or derivatives of these values. From F-statistics and similar approaches, it is possible to estimate the number of migrants between populations per generation. Isolation by distance is also a useful parameter. Assignment tests based on the probability of a genotype occurring in each of a range of populations can allow the identification of individual migrants and their most likely point of origin. The effective size of a population can be difficult to define by ecological methods, but molecular markers facilitate the determination of N_e by various methods (Beebee and Rowe, 2004). Adaptive variation is likely to be important for the long-term viability of a population, highlighting the importance of these kinds of analyses. The genetic difference between populations can be exploited for use in genetic stock assignment techniques, where a mixed group of samples can be assigned back to population of origin. Non-destructive genetic sampling, for example of scales, faeces or hair, is

now becoming widely used in conservation biology as a way of collecting genetic data which helps estimate population census size (Allendorf and Luikart, 2007). Microsatellites are widely used in these kinds of analyses as they have generally high levels of polymorphism (Freeland, 2005).

1.2.1 Evolutionary Implications

Evolution may be defined as the change in the form and behaviour of organisms between generations (Avise, 1994). Evolution is driven by external environmental factors and random genetic changes.

A broad definition of population genetics is the study of naturally occurring genetic differences among spatially distinct conspecifics. Genetic differences between individuals of the same species are genetic polymorphisms, so population genetics is the study of polymorphism and divergence (Hartl, 2000). A population can be defined as a group of potentially interbreeding individuals living within a restricted geographic area differing significantly in genetically determined traits from other populations (Beebee and Rowe, 2004).

Four main drivers which mediate change are mutation, natural selection, genetic drift and migration, all of which can change the genetic composition of populations (Fraser *et al*., 2011). Mutation relates to a change or changes in the DNA of an organism. Mutations generate genetic diversity and mechanisms include point mutation, large deletions and insertions and polymerase slippage at simple sequence repeats. In eukaryotes, mutation rates are usually highest in simple sequences like minisatellites and microsatellites, moderately high in mitochondrial DNA, and lowest in nuclear coding DNA (Beebee and Rowe, 2004). Natural selection, in its directional form, is the deterministic and adaptive process whereby the differential reproduction of individuals of a population results in individuals of a greater fitness producing more offspring than individuals of lesser fitness. Genetic drift is a stochastic process, defined as the random fluctuation of gene frequencies in a population, a factor which is more likely to have an impact on smaller populations (Allendorf and Luikart, 2007). Migration, or gene flow, is the movement of

individuals (and subsequent successful reproduction) between neighbouring populations of a species. If individuals from different aggregations mate freely, there is high gene flow and they remain genetically similar. However, if populations are isolated or gene flow is inhibited for another reason, they can become genetically quite distinct (Fraser *et al*., 2011).

The variation in life history pattern in salmonid species (including both Atlantic salmon and brown trout), and traits such as natal homing and low straying rates lead to strong population structure and are presumed to lead to the development of local adaptation within populations. Kawecki and Ebert (2004), define local adaptation as the expectation of a population having a higher level of fitness in their local habitat than populations from a different habitat, due to genotype x environment interaction impacting on Darwinian fitness. Factors which are expected to promote local adaptation in a species include low gene-flow, e.g. low dispersal or a high level of natal homing, strong selection against genotypes which are adapted to other environments, low rates of temporal variation and differences between habitats in terms of size and quality (Kawecki and Ebert, 2004). Atlantic salmon and brown trout, along with many other salmonid species, are known to exhibit a high level of natal homing with straying rates amongst populations not thought to exceed 4-6%, based on studies in Norwegian rivers (Jonsson and Jonsson, 2003, Vaha *et al.*, 2008). These isolated, sometimes small, populations live in heterogeneous environments (differing temperatures, flow regimes, gravel size) and are subject to different selection pressures (predators, prey, competition, disease) than other conspecific populations, leading to the evolution of optimal strategies for survival in the natal river (Garcia de Leaniz *et al.*, 2007). This usually leads to the development of local adaptation (LA) between genetically isolated populations.

Evidence for LA in salmonids can be divided into several components (Taylor, 1991). These include adaptive variation in morphology (e.g. variations in colour and eye size, (Drinan *et al.*, 2012, Westley *et al.*, 2012)), adaptive variation in behaviour and survival (lifetime success, (Quinn, 1985, McGinnity *et al.*, 2004)), adaptive variation in developmental biology, biochemical and physiological traits (disease resistance MHC variability, (de Eyto *et al.*, 2007, 2011)) and variations in life history (Taylor, 1991).

All of these factors lead to genetic differences between populations, but current evidence for local adaptation in salmonids is considered to be mostly circumstantial (Garcia de Leaniz *et al.*, 2007, Fraser *et al.*, 2011). The criteria required for the demonstration of local adaptation include the expression of varying levels of fitness for different populations across different environments, a higher "local" level of fitness when compared with "foreign" populations and the demonstration that these fitness differences have a heritable component (as detailed in a review by Fraser *et al.*, 2011). These concepts will be discussed further in Chapter Two.

It is however, also believed that salmonid species may vary in their propensity to form locally adapted populations (Quinn, 2005, Fraser *et al.*, 2011). Some salmonids have also demonstrated the ability to be very successful invaders of new environments. Straying between locations during colonisation, is thought to have been very important in establishing the present distribution of salmonid fishes, given that many of their current habitats were ice covered during the last glacial maximum, approx. 14,000 years ago (Bernatchez, 2001, McKeown *et al.*, 2010, Finnegan *et al.*, 2013). The straying of anadromous salmonids is also considered an important factor in the successful invasion of new areas by introduced salmonid species, as considered in Chapter Three (Quinn, 2005, Kinnison, *et al.*, 2008, Launey *et al.*, 2010).

1.3 Atlantic salmon population genetics

Work on the population genetics of Atlantic salmon has focused on several main areas of research. The origins of various races of Atlantic salmon, on both sides of the Atlantic Ocean have been extensively studied (Verspoor *et al.*, 2002, 2005), as has the molecular genetics of Atlantic salmon used in aquaculture (Cross, 1991). Molecular genetic methods have also allowed use of techniques such as genetic stock identification (Kalinowski, 2004, NGSI, 2008) and parentage assignment (McGinnity *et al.*, 1997, and as used in Chapter Two) among others, to be applied to questions, such as the complexities of mating systems and social structuring (Jordan *et al.*, 2007), and local adaptation (McGinnity *et al.*, 2004, Garcia de Leaniz *et al.*,

2007, Fraser *et al.,* 2011). These methods have also been applied in the areas of management and conservation, ranging from looking for evidence of population decline (Conseguera *et al.,* 2007), to the impacts of escaped farmed fish on wild populations (Einum and Fleming, 1997, McGinnity *et al.,* 1997, 2003, Ferguson *et al.,* 2007) to stocking effects (Cross *et al.,* 2007) and effects of fisheries exploitation on populations (Hindar *et al.,* 2007).

It has been shown that Atlantic salmon from the eastern and western Atlantic coasts are two distinct phylogeographic groupings, possibly separate for up to 500,000 years, with only limited gene-flow occurring (Verspoor *et al.,* 2005), suggesting they are divergent enough to be considered as distinct subspecies (Waples, 1991). European Atlantic salmon can be further subdivided into North Atlantic coast and Baltic Sea populations (Stahl, 1987, Nilsson *et al.,* 2001), with further differentiation into regional groupings (King *et al.,* 2007).

Contemporary gene flow among populations has been found to be very restricted in Atlantic salmon, leading to high levels of differentiation between populations, with limited sporadic gene interactions occurring between anadromous populations, linking some of these as metapopulations (King *et al.,* 2007). Temporal samples taken within these differentiated populations can often show little genetic differentiation between years or generations. Atlantic salmon within the same Irish catchment system have shown evidence of population subdivision, partly based on landscape features (Dillane *et al.,* 2008). This suggests that management at the catchment level can risk the loss of much of the diversity within a species or species complex, when these types of variants are not separately acknowledged in management and conservation plans (Waples, 1991).

Stocking of river catchments using transplanted wild and hatchery reared fish is an area of concern, the effects of which has been extensively studied for Atlantic salmon (Cross, 1999, Youngson *et al.,* 2003, Aprahamian *et al.,* 2003). Atlantic salmon have been historically extensively stocked into different rivers throughout their range (Galvin *et al.,* 1996). The questions raised by these practises, such as loss of genetic diversity in the native population, the impact of local adaptation on the success of introduced fish (McGinnity *et al.,* 2004) and the long-term negative

effects of stocking on the native and neighbouring populations have been examined, and are of great importance in terms of conservation and management of the species (Cross *et al.*, 2007). In many cases, efforts to increase natural production in a population, and remove barriers preventing this, are preferable to stocking events, which should only be utilised as a last resort (Cross *et al.*, 2007).

Genetic stock identification (GSI), as used here on brown trout in Chapter Four, was first used on Pacific salmon fisheries as a way of quantifying the proportion of various populations caught in commercial mixed stock fishery. This method allowed managers to estimate the fishing pressure and effect on the conservation limit for particular populations (Kalinowski, 2004). GSI for Atlantic salmon was utilised in the same manner and was applied to several European mixed stock fisheries, e.g. Ireland (NGSI, 2008). Fisheries exploitation of Atlantic salmon has been shown to reduce genetic diversity due to selective removal of a certain part of a population and genetics has shown the effects of fishing on traits such as run-timing and body size (Consuegra *et al.*, 2005, Hindar *et al.*, 2007).

Another method that is useful in population genetics studies is parentage assignment (as used in Chapter Two). This is where a mixed experimental group of fish is created in a hatchery, to answer questions in which knowledge of the parents' source location is important (Hansen *et al.*, 2007). All parental genotypes are known and this allows for offspring to be assigned back to their parents. This allows fish to be "tagged" genetically, so their development and behaviour can be monitored from a much earlier stage than would be possible using external or internal physical tags (McGinnity *et al.*, 1997)

1.4 Brown trout population genetics

Brown trout population genetics has mainly been based on examination of the species' varied life history and the high level of genetic differentiation found between populations. Many lake habitats have been found to support genetically and morphologically distinct brown trout populations, leading to debate about taxonomic definitions within the so-called polytypic species (Waples, 1991, Ferguson, 1989,

2004), which have important considerations relating to conservation and management. The brown trout's high level of variation in terms of life-history patterns expressed within a population, for example, anadromous vs. freshwater resident life history patterns, or the variations seen in freshwater life history patterns is another area where a level of understanding is required for optimal management of the species.

The question why some brown trout remain resident near their spawning area in freshwater, some migrate within freshwater only and some migrate to sea before returning to spawn has been asked by many workers over many years (Nall, 1930, Frost and Brown, 1967, Ferguson, 1989, Poole *et al.*, 2006, Gargan *et al.*, 2006). Brown trout can exhibit various types of freshwater forms as adult fish, such as river resident, lake-resident, and ferox types (Frost and Brown, 1967). Sea trout are an anadromous form of brown trout (*Salmo trutta* L.), meaning that they spend a period of their life history feeding in the marine environment before returning to freshwater to reproduce. Some brown trout, known as slob trout migrate as far as the estuarine environment, but do not migrate to full salinity waters (Harris and Milner, 2006). These various types can all be described as forming part of the brown trout species complex (Ferguson, 2004).

Studies examining different tributary groups of brown trout usually showed significant genetic differentiation, with assortative mating being common in trout populations, for example, within a lake system (Ferguson, 2006). There are several examples, both in Ireland and internationally, of genetically distinct populations of brown trout occurring in the same catchment (Ryman *et al.*, 1978, Ferguson, 1989, Duguid *et al.*, 2006, Massa-Gallucci *et al.*, 2010, Prodohl *et al.*, unpublished report).

As was mentioned in the previous section on Atlantic salmon, spatial samples, such as brown trout sampled from different catchments, or from tributaries within the same catchment, often show significant levels of genetic differentiation. Brown trout populations are thought to show more population structuring than Atlantic salmon populations (Ferguson, 2006). Possibly the fact that trout are more genetically divergent than Atlantic salmon and show more phenotypic plasticity and a greater

range of life history variation could be part of the reason for this difference (Ferguson, 2004, Verspoor, 2007, Westley and Fleming, 2011).

Irish brown trout populations from neighbouring catchments have been shown to be significantly different from each other (Fahy, 1985, Poole *et al.,* 2006). Population structuring has been commonly observed within catchments, with, for example, Loughs Corrib and Mask in western Ireland showing evidence of population structuring (Ferguson, 2004, Massa-Gallucci *et al.,*, 2010, Prodohl *et al.,* unpublished report). The Burrishoole catchment in western Ireland has also shown evidence of population structuring of brown trout within a catchment (Poole *et al.,* 2006). Temporal samples taken within populations generally show lower, or no, levels of differentiation, suggesting these genetic differences between population groupings are likely to be due to natal homing and low levels of straying between populations. Population differences have also been found on either side of impassable barriers, e.g. waterfalls (Cross *et al.,* 1992, O'Farrell *et al.,* 2012).

Lough Melvin in north-western Ireland has been extensively studied in terms of its brown trout population genetics (Ferguson and Mason, 1981, Ferguson, 1989, Cawdry and Ferguson, 1988 and McVeigh *et al.,* 1995). This lake has a unique trout community, supporting three sympatrically feeding forms of brown trout: namely gillaroo, sonaghen and ferox, known to differ morphologically, by feeding habits, and also genetically (Ferguson, 2004). This distinction between trout forms in Lough Melvin is known to be currently maintained by separation of spawning sites due to natal homing (Ferguson, 1989, 2006). Lough Neagh, in Northern Ireland, has two distinct types of brown trout, the dollaghan and the so called "salmon" trout, which are genetically distinct and breed in separate rivers (Crozier and Ferguson, 1986). In Scotland, Lochs Awe and Laggan are found to have populations of ferox brown trout along with sympatrically feeding populations of genetically-distinct brown trout (Duguid *et al.,* 2006), while Ryman *et al.,* (1978) found two co-existing populations of genetically distinct brown trout in a Swedish lake. These examples are thought to represent within-lake structuring, which has evolved since the most recent colonisation of the habitats after the last ice age, leading to the creation of distinct brown trout forms. The exception to this is ferox trout, which are believed to be descended from a separate lineage and are described as a separate species, *Salmo*

ferox, by the IUCN (Freyhof and Kottelat, 2008). Ferox trout are found in several areas across the islands of Ireland and Britain and share traits not usually observed in brown trout, such as longevity and piscivory (Fahy, 1985). Genetically, the ferox trout is also much differentiated from other brown trout forms (Duguid *et al.*, 2006).

Anadromous brown trout migrations are thought to be triggered by a combination of genetic and environmental factors (Ferguson, 2006). This is thought to be an example of a threshold quantitative trait, that is, it is expressed once a combination of factors reaches a threshold level, triggering anadromy. Anadromy is believed to be the ancestral state for this species, and freshwater resident forms of brown trout have evolved from anadromous ancestors at some stage over the past 14,000 years (Bernatchez, 2001, McKeown *et al.* 2010), which suggests that adaptation to an entirely freshwater lifecycle occurred independently in each catchment. It is also now recognised that the freshwater resident life-history has possibly arisen more than once in the same catchment since the last glacial maximum, leading to sympatric, genetically isolated populations of resident brown trout (Ferguson, 2004, 2006, McKeown *et al.*, 2010).

Previous work has shown the genetic basis to the physiological changes that sea trout undergo before migration to sea. These include changes in gene expression during smoltification, and the retention of the ability to smoltify in populations that have been unable to migrate to sea for thousands of generations (Foote *et al.*, 1994, Dann *et al.*, 2003, Giger *et al.*, 2006). Migration itself seems to be under genetic control and has a high heritability (Fleming, 1983, Jonsson *et al.*, 1994, Ferguson, 2006, Poole *et al.*, 2006). Work on the response of brown trout in the Burrishoole catchment, western Ireland, to increased levels of marine mortality of smolts suggests marine migration in the Burrishoole catchment is mainly under genetic control (Poole *et al.*, 2006). The high marine mortality of smolts, beginning in 1989, resulted in low numbers of adult sea trout returning to the catchment which was followed by a collapse in smolt output. In this case it has been concluded, that the spawning of an adult sea trout phenotype is needed to produce sea trout smolts (Poole *et al.*, 2006). The prevalence of anadromous trout seems to increase with decreasing opportunities for growth in the freshwater environment, so less productive systems are generally thought to produce a higher proportion of sea trout,

with a gradient of increasing anadromy occurring as you move north in Europe (Jonsson and Jonsson, 1993, Elliott, 1994, Poole *et al.,* 2006). Genetic studies examining differences between sea trout and resident morphs of the same populations have generally found no significant differences if cohabiting, implying random mating (Hindar *et al.,* 1991, Pettersson *et al.,* 2001), and suggesting interbreeding occurs between the two forms.

Historically, brown trout have been stocked across rivers and lakes in Ireland, and in other European countries, ostensibly to increase catches for anglers. The Howietoun hatchery in Scotland, supplying the Loch Leven strain, was the source for many of these stocking events and also the source of the brown trout introduced to Newfoundland, Canada, as discussed in Chapter Three.

Quantitative genetics focuses on traits, such as growth rate, egg size, temperature tolerance, which are influenced by many genes and by environmental factors, is also an important area of research in Atlantic salmon and brown trout (Garcia de Leaniz *et al.,* 2007). With recent advances, information is becoming available on the genes that influence specific traits (Hansen *et al.,* 2007). Particularly, for issues such as the genetic control of anadromy in brown trout, this kind of approach could allow for the unravelling of still-poorly understood mechanisms. Quantitative trait loci (QTLs) can be assessed statistically to measure their effect. The future of Atlantic salmon and brown trout population genetics, in light of recent advances in the field of genomics, will likely involve the integration of the areas of population genetics and quantitative genetics (Hansen *et al.,* 2007).

1.5 Invasive Species

Biological invasions have been recognised as second only to loss of habitat and landscape fragmentation as major causes of loss of global biodiversity (Allendorf and Luikart, 2007). An invasive species can be defined as a non-native species, introduced to a new environment through human actions, which has spread without human input and is known to cause ecological damage in the new environment (Lockwood, 2005, Estoup and Guillemaud, 2010). There are several possible

mechanisms involved in a human-mediated biological invasion. These include intentional introductions for reasons such as pest control (or, as in the present case, the development of a fishery) to accidental introductions through commercial transport and shipping (Estoup and Guillemaud, 2010).

It has been argued that biological invasions are just examples of natural range expansions of species (Vermeij, 2005), such as those that occurred in the natural recolonisation of previously glaciated areas of Europe by, for example, brown trout after the last glacial maximum (McKeown *et al.,* 2010). Invasions, however, often involve multiple source populations, multiple introduction sites and can occur much more rapidly than natural colonisation or recolonisation by species (Estoup and Guillemaud, 2010). This can result in the introduction of a great deal of genetic variation over a short time period (Roman and Darling, 2007, Wilson *et al.*, 2009). This could be advantageous for an invasive species adapting to novel environmental conditions, thus increasing the chance of adaptive genetic variants being included amongst the founders and also increasing the potential of offspring to respond to natural selection (Roman and Darling, 2007). One of the main reasons why invasions involve multiple sources and sites, and occur rapidly is because of human mediated factors, either, as outlined above, intentional, such as introducing new species to an area or unintentional, by providing vectors for dispersal, e.g. boats, train networks, canals or by the modification of natural environments (Estoup and Guillemaud, 2010).

Reconstruction of the routes and patterns of invasions can be important for designing strategies to prevent and mitigate against invasions. For example, if the origin and method of transport can be identified in the case of a recurring unintentional invasion, steps can be put in place to prevent its re-occurrence (Mack *et al.,* 2000). Also, in terms of slowing the spread of an already established invasive species, understanding the pattern of geographic spread can be useful. An understanding of the routes and patterns of invasions is also important for defining and testing hypotheses relating to environmental and evolutionary factors in biological invasions (Estoup and Guillemaud, 2010). These include differentiating between neutral phenotypic changes and adaptive evolution in invasive populations, and trying to separate out the role of stochastic and deterministic events in the population (Keller

and Taylor, 2008). In this thesis, the term invasive "population" will be used for largely or completely isolated aggregations, which genetic analyses have shown to differ significantly in composition from other such aggregations (i.e. largely separate breeding units). It is recognised however, that such entities may not yet have achieved genetic equilibrium, such as is observed in natural populations within the native range.

It has been estimated that only one in ten attempted invasions are actually successful in establishing a species in a new environment (Allendorf and Luikart, 2007). Current research to understand the reasons for successful invasions into new environments focuses on phenotypic traits such as wide dispersal capability, high growth rate, high level of competiveness and tolerance of environmental heterogeneity (Sakai *et al.*, 2001, Kolar and Lodge, 2002). However, it has been found that invasiveness can vary between species with apparently similar characteristics, as well as within species (Kolar and Lodge, 2002, Allendorf and Luikart, 2007). A review by Hanfling (2007) highlights the importance of understanding the phenotypic and genetic characteristics of successful invaders, as well as their invasion pathways. These factors contribute to many aspects of the management of non-native invasive species and conservation of native species that are threatened by invasives. These include assessment of ecological and evolutionary effects on recipient ecosystems, and possible benefits, as well as the development of protective legislation (Hanfling, 2007).

Population genetic theory predicts that the amount of genetic variability in a species, compared with that occurring within its natural distribution, determines its ability to adapt to new or changing environmental conditions (Hanfling, 2007, Blanchet, 2012). New environmental conditions encountered by introduced species may be considerably different from source conditions. Therefore, natural selection and adaptation may be important factors for successful invasions (Estoup and Guillemaud, 2010). High levels of genetic variability are thought to favour invasion success in species entering new and different environments. However, there are also examples of successful invaders which exhibited very low levels of genetic variability, such as the Argentine ant (*Linepithema humile*) (Tsutsui *et al.*, 2000, Estoup and Guillemaud, 2010). It has been theorised that advantageous alleles or

advantageous genotype combinations for those alleles may facilitate successful introductions in these cases (Facon *et al.*, 2006).

The two main approaches for investigating routes of invasion, according to a review by Estoup and Guillemaud (2010), are by using direct methods, i.e. based on observations and records, and indirect methods, which are based on patterns in population genetics data. Direct methods can be useful but may be inaccurate, incomplete or based on inaccurate information. Indirect methods using genetic data have been widely used (e.g. Kinnison *et al.*, 2002, Kinnison *et al.*, 2008, Valiente *et al.*, 2010a, 2010b, Launey *et al.*, 2010) and include using population structure analysis to determine routes of invasion (see below).

As stated above, knowledge of invasive species is frequently based on historical observational data which can be sparse, incomplete and misleading. Population genetics can therefore prove to be a useful approach for determining the routes and patterns of invasion (Estoup and Guillemaud, 2010). With increasing research into the topic, it is becoming clear that evolutionary processes play an important role in the establishment success of non-native species. Genetic drift during colonisation followed by strong selection pressures in a novel environment, and also co-evolutionary disequilibrium can provide the conditions for rapid evolutionary change in introduced populations (Hanfling, 2007). Invasive fish species can change community structure by outcompeting or hybridizing with native fish fauna, as well as having possible "top down" effects on lower trophic levels, such as benthic fauna and phytoplankton and zooplankton (Townsend, 2003, Hanfling, 2007).

Molecular genetic analysis of an introduction can provide information, if required, on the possible control of existing invasions and future prevention of new invasion events (Mack *et al.*, 2000). The source of the invading species, the number of invasion events that occurred, the size of the original introduced population and the subsequent pattern of spread are important factors for determining effective control measures. Genetics can also be used to help determine which species are likely to become successful invaders. To invade an ecosystem successfully, a species must arrive, survive and establish itself, before spreading and replacing or outcompeting native species. Several genetic principles that help predict which species may be

successful invaders, are the extent of genetic drift and the effects of small populations, and the amount of gene flow, natural selection and adaptation (Allendorf and Luikart, 2007). Figure 1 shows some of the main theoretical considerations relating to an invasion event.

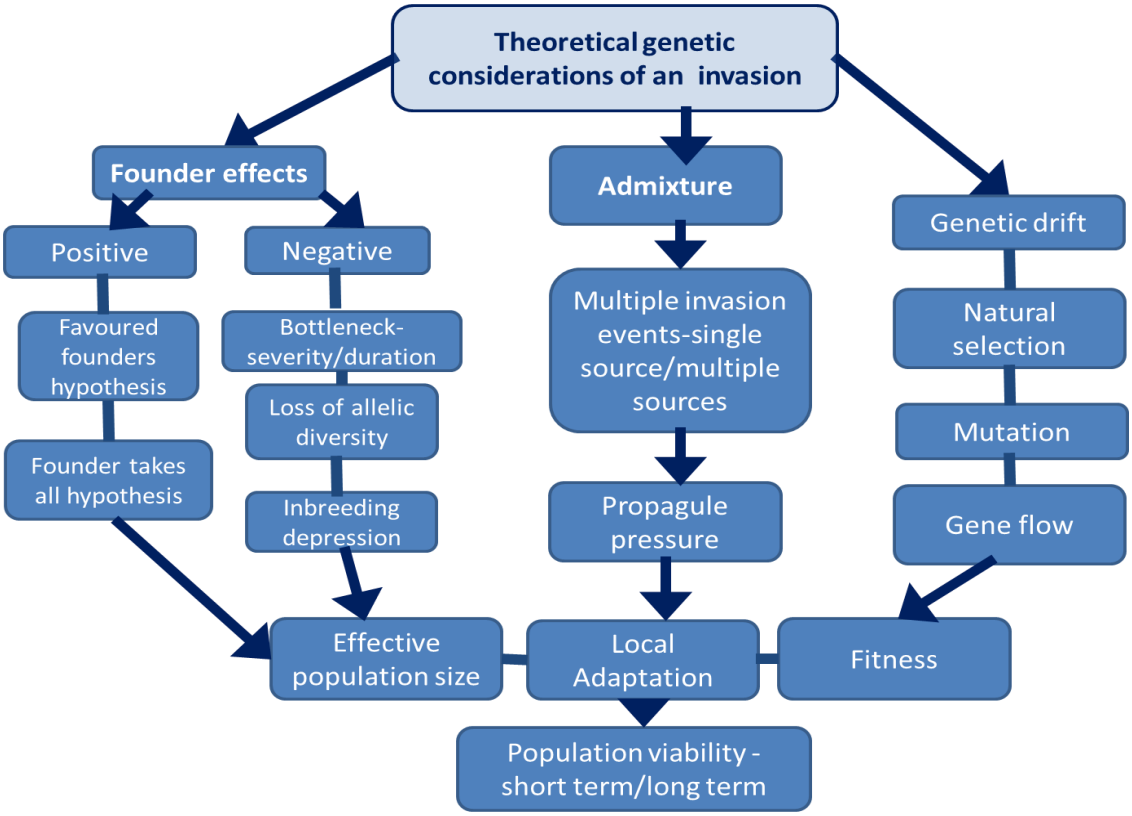

Figure 1: Diagram showing some of the main theoretical genetic considerations relating to invasion events (see text for details).

Invasive species frequently go through a bottleneck during their establishment period in a new environment, resulting in a substantial loss of genetic diversity. The most severe population bottleneck occurs where only one male and one female produce offspring for future generations. Founder effects occur where the founding of a new population by a small number of individuals (a form of bottleneck event) causes changes in allele frequency and loss of genetic variation. This frequently results in the loss of rare alleles, with the effect being more pronounced after a severe bottleneck event. Thus, founder effects/bottlenecks will result in a reduction in the overall number of alleles in a population (Barrett and Kohn, 1991, Allendorf and Luikart, 2007). Such a reduction in allele numbers and loss of rare alleles can have a

knock-on effect of the invading species' fitness and resilience, and therefore its long term viability. Inbreeding depression can limit population growth, and low genetic variation limits the species ability to adapt to their new environment (Beebee and Rowe, 2004). This raises the first of two supposed genetic paradoxes relating to invasive species. If population bottlenecks are harmful, why are invasive species that have gone through a bottleneck, successful? Several theories have been put forward to explain this paradox. Firstly, introduced species are frequently more genetically diverse than native species as they can be sourced from several distinct populations (Allendorf and Luikart, 2007), so a bottleneck would have a less severe effect on their genetic variability than in a less diverse population (although this scenario may in turn result in issues with outbreeding depression). Rare alleles can also survive bottlenecks to become more common after the bottleneck, resulting in an increase in adaptive evolutionary potential (Hanfling, 2007, Waters *et al.,* 2013), often referred to as allele surfing.

Propagule pressure is known to have a strong effect on the success of an invasion. That is, the larger the number of individuals introduced and the higher the incidence of invasion events, the better the chance of success of an invasive species (Allendorf and Luikart, 2007).

In freshwater systems, intentional releases of non-native fish species have been common practise for commercial and recreational purposes (Hanfling, 2007). These introductions provide an opportunity to study other species reactions to colonisations and their adaptation to novel environments (Sax *et al.,* 2005, Westley and Fleming, 2011, Blanchet, 2012). The management of freshwater systems which have been invaded by non-natives, or which are at risk of invasion, is complicated by several factors. These include the perception of the invasive and the perceived 'worth' of the impacted native fauna (Young *et al.,* 2009, 2010). In the southern hemisphere, for example, some invasive salmonid species (*Salmo trutta,* brown trout and *Oncorhynchus mykiss*, rainbow trout) are highly valued angling species, generating tourism revenue, while other species, or the same species in a different region, can be regarded as a threat to native fauna and biodiversity (Garcia de Leaniz *et al.,* 2010).

An example of a highly successful salmonid invasive is the brown trout, which was introduced extensively worldwide during the late 19th century as it was prized by anglers who had left Europe to settle in various places around the world (Westley and Fleming, 2011). In the northern hemisphere, on the North American continent, there has been evidence of successful introduced brown trout populations negatively impacting on native populations of brook trout (*Salvelinus fontinalis*) and Atlantic salmon (*Salmo salar*, O'Connell, 1982, Westley *et al.*, 2012). Atlantic salmon were also extensively introduced into the wild in several regions worldwide but have not shown the level of success in establishing new populations as brown trout, even in areas where they have been introduced as conspecifics (Ayllon *et al.*, 2006). As will be discussed in Chapter Three, the genetics of invasive brown trout populations have also been studied in some regions where they have been successfully introduced. Work in the Kerguelan Islands (Launey *et al.*, 2010), where there are no native fish fauna, and in South America (Valiente *et al.*, 2010, Young *et al.*, 2010), where brown trout have had a negative impact on native fish species, show the brown trout's ability to successfully invade and colonise a new environment.

1.6 Management and Conservation

As mentioned in previous sections, much of the work carried out on the population genetics of Atlantic salmon and brown trout has important implications for the management and conservation of these species. The main threats to the continuing viability of existing native populations of both species are broadly similar, including habitat destruction and degradation, impacts of stocking, invasive species and the effects of aquaculture (Harris and Milner, 2006, Verspoor, 2007). These threats can lead to decline in population size, loss of genetic diversity and local adaptations and therefore cause a decline in overall species resilience.

Atlantic salmon and brown trout are economically important species in many areas, although Atlantic salmon is by far the most important species of the two in terms of aquaculture (Verspoor *et al.*, 2007). Brown trout are farmed less widely, but are extensively reared for stocking (Ferguson, 2004). Angling tourism revenue is also important financially in regions with populations of these species (Ferguson, 2004).

Atlantic salmon are regarded as important species in a conservation context, and are protected in freshwater under the EU's Habitats Directive (Council Directive 92/43/EEC), whereas brown trout are excluded from the Directive. Brown trout can cause a conservation issue outside of their native range as a successful invasive species, and are regarded as a pest in many areas (Westley and Fleming, 2011).

1.7 Background to Project

This PhD research was funded as part of the Beaufort Award in Fish Population Genetics, a seven year programme under the Irish National Development Plan. The Beaufort Fish Genetics group is a collaborative group between researchers in fish population genetics in the School of Biological, Earth and Environmental Sciences, University College Cork and the School of Biological Sciences, Queens University Belfast. This PhD was divided into three main areas which are presented here as separate chapters.

1.8 Main Hypotheses of the Thesis

The overarching objective of the thesis is to explore various aspects of biological significance of genetic variation in Atlantic salmon and brown trout by way of addressing a series of ecological and evolutionary biology questions, summarised below. Individual project goals are described in greater detail in each of Chapters Two, Three and Four.

Chapter Two examined the hypothesis that the Atlantic salmon (*Salmo salar*) population from the Burrishoole catchment is locally adapted to its native catchment and will show greater survival rates than transplanted salmon from the neighbouring Owenmore catchment. Various studies dispute the idea that local adaptation in salmonids occurs at small geographical distances (Adkison, 1995, Fraser *et al.,* 2011 and Meier *et al.,* 2011). This study aimed to examine local adaptation, in terms of survival and lifetime success, of neighbouring Atlantic salmon populations to demonstrate the existence of biologically important local adaptations at small geographical scales. A previous common garden study in the west of Ireland

(McGinnity *et al.* 2004) had demonstrated substsntially lower survival from egg to returning grilse in salmon transplanted as eggs from the nearby Owenmore River compared with Burrishoole River natives. The present study sought to determine whether such differences were relatively constant over time and, by including inter population hybrids which had not featured previously, whether there was an additive genetic basis for such differences (i.e. whether there was evidence of local adaptation).

Chapter Three examined the invasive brown trout populations of the Avalon Peninsula, Newfoundland, Canada. One of the main hypotheses examined in Chapter Three, was that genetic analysis would confirm the expected route and means of invasion used by the invading brown trout over the past 130 years, as indicated by historical records and ecological studies (Westley *et al.*, 2011, Westley and Fleming, 2012). This study also proposed the hypotheses that the majority of the current population of brown trout in Newfoundland were descended from Scottish hatchery fish, sourced from the Howietoun hatchery, and introduced into one area of Newfoundland from the 1880's.

The main hypothesis of Chapter Four was that the long-lived large sea trout found in Lough Currane could be assigned back to a river or region of origin within the Currane catchment using individual assignment analysis (IA). This was based on the hypothesis that population structuring would be observed between different rivers and sub-catchments within the Currane catchment, located in Co. Kerry, in the south-western corner of Ireland. While GSI and IA analysis has been carried out previously on Atlantic salmon and resident brown trout populations in Ireland, no work has been done up to now on sea trout intra-catchment population variation.

These three data chapters look to examine different areas of genetic population structure of Atlantic salmon and brown trout in both Ireland and Newfoundland, with the overall aim of adding to our current knowledge on how salmonid populations act both between and within catchments.

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Chapter 2:

Investigating local adaptation in *Salmo salar*, L.: assessing the relative fitness of native and non-native salmon in a common garden experiment in the wild.

Srahrevagh River fish trap, Burrishoole catchment (Photo courtesy Ger Rogan).

Abstract

This common garden study with Atlantic salmon, *Salmo salar* in the Burrishoole system in western Ireland, was designed to assess the mode of genetic control of performance traits. Parentage was assigned using molecular techniques (microsatellite loci). The freshwater phase of the experiment consisted of an in-stream common-garden experiment, with eyed eggs from the four study groups (pure natives, female native x Owenmore male, female Owenmore x native male, pure Owenmore) being outplanted to an enclosed stretch of river, while hatchery reared smolts from each of the four groups were released to sea. The overall lifetime success of the offspring of Owenmore parents in this experiment, including both freshwater and marine phases was 35% compared with native Burrishoole fish. This result is similar to the 38% value of non-native Owenmore to native Burrishoole survival observed in a previous experiment with the same polulation in the experimental stream a decade earlier. The marine phase, rather than the freshwater was found the major determinant of survival here, as in the previous study. In this experiment, the relative performance of hybrids was also assessed. There was no evidence of hybrid vigour (heterosis) in survival. Instead, the survival of the hybrid groups was largly intermediate between the levels observed for native and Owenmore salmon indicating additive genetic variation. There were quantitative differences between native and introduced fish in behavioural traits such as juvenile dispersal and timing of adult return to catchment, with mid-parent values being indicative of genetic control for these life history characters. The results of this new experiment support the idea of local adaptation in salmon occurring at geographical scales of less than 50km, with evidence of a substantial degree of mal-adaptation (65 to 75%) in the offspring of non-native fish even for regionally proximate populations. These results provide important insights into the operation of local adaptation in salmon, and may have important implications for the management of salmon populations.

2.1 Introduction

Local adaptation (LA) results from the genetic isolation and divergence of populations in different habitats, where divergent selection exceeds the effect of gene flow and random drift (Kawecki and Ebert, 2004, Gharrett *et al.,* 2013). It can be viewed as the "end product of natural selection" (Garcia de Leaniz *et al.,* 2007), representing the degree of matching between phenotype and environment. Factors thought to encourage the development of LA in a population include: low gene flow, a greater amount of spatial than temporal variation in the forces of selection within a species, small changes in habitat quality and where constraints to plasticity exist within an environment (Kawecki and Ebert, 2004, Fraser *et al.*, 2011). Selection can be negated by gene flow or made less efficient by genetic drift, so LA is not necessarily an inevitable outcome, even where different selective processes are known to operate (Wright, 1931, Fraser *et al.*, 2011). Local adaptation and its genetic basis are reviewed in more detail in Chapter 1.

Atlantic salmon and brown trout along with other salmonids, are considered suitable species for examining adaptive variation for several reasons: they have high fecundities, inhabit a number of very different habitats from temperate to Arctic regions, and tend towards natal homing, all characteristics, which are thought to promote development of locally adapted populations (Allendorf and Waples, 1996, Garcia de Leaniz *et al.,* 2007). LA in salmonid fishes is thought to be very important for the overall resilience and productivity of species. Work carried out on sockeye salmon (*Oncorhynchus nerka)* populations in the Bristol Bay area of Alaska has shown the importance of populations being adapted to their local habitat (Hilborn *et al.,* 2003, Schindler *et al.*, 2010).

LA in salmonids can be tested for, and has been demonstrated in the past, by using a large variety of methods and approaches, which include: examining differences at the phenotypic or molecular level and correlating these with environmental factors (Bernatchez, 2004, Waples, 2004, Fraser and Bernatchez, 2005, Quinn, 2005, Vaha *et al.,* 2008), looking at genetic differentiation in traits possibly exposed to selection relative to neutral markers e.g. MHC studies (de Eyto *et al.,* 2007, 2011, Conseguera

and Garcia de Leaniz, 2008) and elucidating the molecular basis for trait adaptation (Goetz *et al.*, 2010). More environmentally-based approaches which involve the manipulation of natural populations to test for trait or fitness differences have also been employed in previous studies (Einum and Fleming, 2000, McGinnity *et al.*, 2004, Quinn, 2005, Kinnison *et al.*, 2005, Westley *et al.*, 2013). It is generally agreed that these approaches provide some evidence of LA in salmonids across a range of spatial and temporal scales. However, it is also agreed that these approaches still lack definitive proof of LA in salmonids (Endler, 1986, Taylor, 1991, Adkinson, 1995, Fraser *et al.*, 2011). Previous reviews have called for more robust or empirical testing of LA in salmonids (Taylor, 1991, Quinn, 2005, Garcia de Leaniz *et al.*, 2007, Fraser, *et al.*, 2011 and Hutchings, 2011). Suggestions mainly involve looking at the extent and scale of LAs, for example: further work on the heritability of traits related to fitness: examination of the extent of phenotypic plasticity and genotype x environment interactions, and identification of what agents of selection are most important (Taylor, 1991, Kawecki and Ebert, 2004, Fraser *et al.*, 2011). Reciprocal transfer and common garden experiments in the wild have been recommended by several reviewers to unequivocally test for LA (Taylor, 1991, Kawecki and Ebert, 2004, Garcia de Leaniz *et al.*, 2007, Fraser *et al.*, 2011). These experiments should be capable of discriminating between the effects of phenotypic plasticity and additive genetic effects so as to uncover or rule out LA. These experiments can be conducted in either captivity or in the wild, but preferably the latter. Carrying out experiments in the wild removes any artificial effects of hatchery rearing. It is important to introduce the populations as early in the life-history as possible, to reduce selection and hatchery effects. Replication of experiments has also been highlighted as an important factor in substantiating the evidence of divergent selection, rather than as a result of random events in a population's history (Kawecki and Ebert, 2004).

A review and meta-analysis by Fraser *et al.* (2011) suggested that the LA of salmonid fishes could be observed across several spatial scales, with LA occurring more frequently and with greater strength as geographic distance increases. Adkison (1995) disputed the effect of salmonid LA over small geographic distance (< 50km). Recent studies, however, (McGinnity *et al.*, 2004, Meier *et al.*, 2011, Gharrett *et al.*,

2013, Westley *et al.*, 2013) provide evidence of salmonid LA occurring across smaller geographic distances (50km or less).

The inclusion of hybrid genotypes has also been proposed as an important consideration in disentangling genetic from environmental effects by several authors in reciprocal and common garden studies (Hatfield and Schluter, 1999, McGinnity et al. 2003, Kawecki and Ebert, 2004). LA is characterised by the inferiority of immigrants relative to local inhabitants, and it is theorised that hybrids between immigrants into a locally adapted population will display a fitness that could deviate from an average of the two parental genotypes (Kawecki and Ebert, 2004). First generation hybrids may display hybrid vigour (heterosis) (Gilk *et al.*, 2004), or extrinsic outbreeding depression, while hybrids of second or later generations could suffer from outbreeding depression associated with intrinsic incompatibilities (Edmands *et al.*, 2002, Bryden *et al.*, 2004). Hybridisation between distinct populations and introgression of non-native genes can erode the fitness of native populations through outbreeding depression, either by producing a phenotype which is intermediate to both contributing genomes, which would be maladapted to both source environments, or by disrupting distinct co-adapted and inter-linked complexes of epistatic genes, i.e. the organism's genetic architecture (Gilk *et al.*, 2004). Studies have shown that hybrids between farmed and wild Atlantic salmon have intermediate or lower fitness than wild fish (McGinnity *et al.*, 2003). Similarly, hybrids between native and non-native pink salmon (*Oncorhynchus gorbusha*) also have shown reduced fitness (Gilk *et al.*, 2004). F1 hybrids have been previously shown to demonstrate intermediate distributions relative to parental populations in the marine environment: (Brannon and Hersberger, 1984, Kallio-Nyberg *et al.*, 2000, McGinnity *et al.*, 2003).

Conservation and management of salmonid populations is greatly influenced by considerations of LA, particularly as understood within the concept of the 'precautionary principle'. The 'precautionary principle' states that if an action or policy has a suspected risk of causing harm to the environment, in the absence of scientific consensus that the action or policy is actually harmful, then the burden of proof that it is not harmful falls on those taking the action (NASCO, 1998). An ability to quantify the scale and extent of LA is therefore important for defining and

applying conservation units within species, as well as applications in conservation prioritization and in restoration issues (Waples, 2004, Fraser *et al.*, 2011).

2.1.1 Main Hypothesis:

This study examined the hypothesis that the Atlantic salmon (*Salmo salar*) population from the Owenmore catchment will show no difference in survival rates or behaviour from natives when transplanted into the neighbouring Burrishoole catchment. Various studies dispute the idea that local adaptation in salmonids occurs at small geographical distances (Adkison, 1995, Fraser *et al.*, 2011 and Meier *et al.*, 2011). This study aimed to examine local adaptation, in terms of survival and lifetime success, of neighbouring Atlantic salmon populations to demonstrate the existence of biologically-important local adaptations at small geographical scales. A previous common garden study in the same experimental stream with the same two populations (McGinnity *et al.* 2003) had demonstrated dramatically lower survival from egg to returning grilse in salmon transplanted as eggs from the nearby Owenmore River compared with Burrishoole River natives. The expectation is that if LA exists, the native population will have a higher survival rate than the introduced fish, and that hybrids will have intermediate fitness relative to the offspring of the pure parental crosses (Burrishoole and Owenmore). The inclusion of inter-population hybrids in the current study is designed to provide a definitive conclusion with respect to the genetic basis of any differences in the biology and fitness that may be observed between the native and non-native fish.

2.2 Methods

2.2.1 Experimental populations and their source locations

The Burrishoole system, situated in Newport, Co. Mayo, consists of some 45km of rivers and streams, which are all very spate-like in nature, rapidly rising and falling in response to precipitation. These rivers drain into Lough Feeagh, the largest freshwater lake of the catchment (320 ha). Two outlets from Lough Feeagh feed the tidal Lough Furnace (141 ha), both of which have permanent upstream and downstream trapping facilities maintained by the Marine Institute, for counting fish migrating between the sea and freshwater. Lough Furnace then flows into Clew Bay. The Marine Institute's facility in Burrishoole is an international index site for the diadromous species, Atlantic salmon, brown trout and the eel (*Anguilla anguilla*). Other fish species present in the Burrishoole catchment are the Arctic charr (*Salvelinus alpinus*) and three spined stickleback (*Gasterosteus aculeatus*). In addition to the fish-stock monitoring programme, a comprehensive environmental monitoring network exists (Whelan *et al*., 1998). Total trapping of downstream salmon and sea trout smolt migrations have been carried out on the Burrishoole system since 1970. The traps have enabled a census of all migratory salmonid movements upstream and downstream since 1971 (Poole *et al*., 1996).

The Burrishoole system has a run of both grilse or 1 sea-winter (1SW) and 2 sea-winter (2SW) salmon. There is only a small multi-sea winter component in the Burrishoole stock, normally <10% of the total run. The numbers of Atlantic salmon recorded in the upstream traps since full trapping commenced in the 1970s have fluctuated from a high of 1,777 in 1973 to a low of 252 in 1990. Annual escapement to freshwater is known to have been influenced by both the level of exploitation in the coastal drift net fishery and fluctuation of marine survival to the coast. Following the cessation of the drift net fishery in 2007, a marked increase in the numbers of fish returning to the catchment was recorded in that year. However, this increase occurred only in 2007, with numbers in 2008 and 2009 falling to levels similar to those recorded prior to the cessation of drift netting. This fall in numbers would

indicate a probable decrease in marine survival in line with the reported downward trend in pre-fishery abundance for Irish populations (Peyronnet *et al.*, 2008).

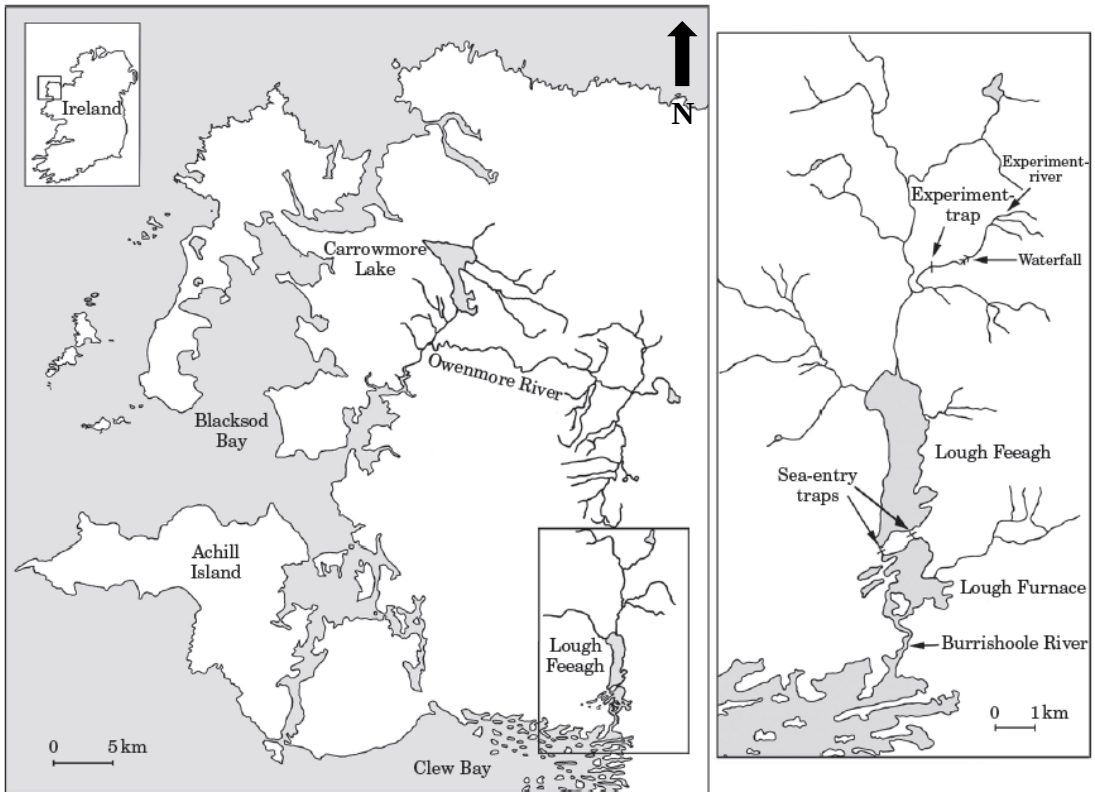

Figure 1: The Burrishoole and Owenmore catchments and the location of the Srahrevagh River trap, the Mill Race trap and the Salmon Leap trap within the Burrishoole catchment.

The neighbouring Owenmore River consists of a number of similar spate-like rivers and streams, which feed into the main Owenmore River. The Owenmore catchment drains directly into the sea. The river, which flows through part of north Mayo, joins the Atlantic Ocean at Tullaghan Bay, just to the north of Achill Island (Figure 1). Brown trout, eel and stickleback are also present in the Owenmore catchment. The Owenmore River is a large spate river, the main channel of which is approximately 16km in length, with approximately 50km of afferent tributaries, and is renowned as a salmon angling river, with a run of both 1SW and 2SW salmon. There is no history of aquaculture in the Owenmore River or the immediate estuary. The Owenmore catchment is not as intensively monitored as the neighbouring Burrishoole, although Inland Fisheries Ireland maintains a fish counter on the river to estimate adult salmon run annually. The mouth of the Owenmore River is approximately 50km (in coastal distance) from the outflow of Lough Furnace in Clew Bay. Both catchments share similar geology, although land use is different in the two areas. The

Burrishoole catchment is partially forested and some farming occurs, while the Owenmore River flows through bog for a large portion of its length. Both catchments have their source in the same part of the same mountain range in north Co. Mayo, with tributaries rising within 0.5km of each other.

Adult salmon, used as broodstock in this experiment, were sourced from both the Owenmore and Burrishoole catchments during the spawning season of 2008. Mature adult salmon were collected from the Owenmore River by electrofishing during November 2008, and held at facilities within the catchment on the Glencullin River. Returning mature adults to the Srahrevagh River, within the Burrishoole catchment, were collected using the trapping facilities present on the river (Figure 1). Age and size of the broodstock used are detailed in Appendix I. This manner of broodstock collection follows that used by McGinnity *et al.* (2004), with one difference. In the winter of 2008, while an attempt was made to collect only 1SW fish, some 2SW fish were used, due to a lack of available 1SW broodstock (see details in Appendix I).

Experimental Site

The freshwater phase of this experiment took place in the Srahrevagh River (Figure 1, Figure 2). This third order stream is located within the Burrishoole catchment. The experimental site is a 2km stretch of stream, consisting of 7,250 m^2 of salmonid habitat, bordered at one end by a series of impassable waterfalls and at the other end by a fish trap capable of catching all downstream and upstream migrants from alevins to adult broodstock over a wide range of stream flows. The trap consisted of a double screen system of interchangeable steel plates ranging in mesh size from 2mm to 8mm set obliquely across the stream. Fish meeting the screens are diverted into a 50m x1m x 1m channel, which feeds into a horizontal incline screen before entering a submerged fine mesh holding box (Wolf, 1951, McGinnity *et al.*, 1997).

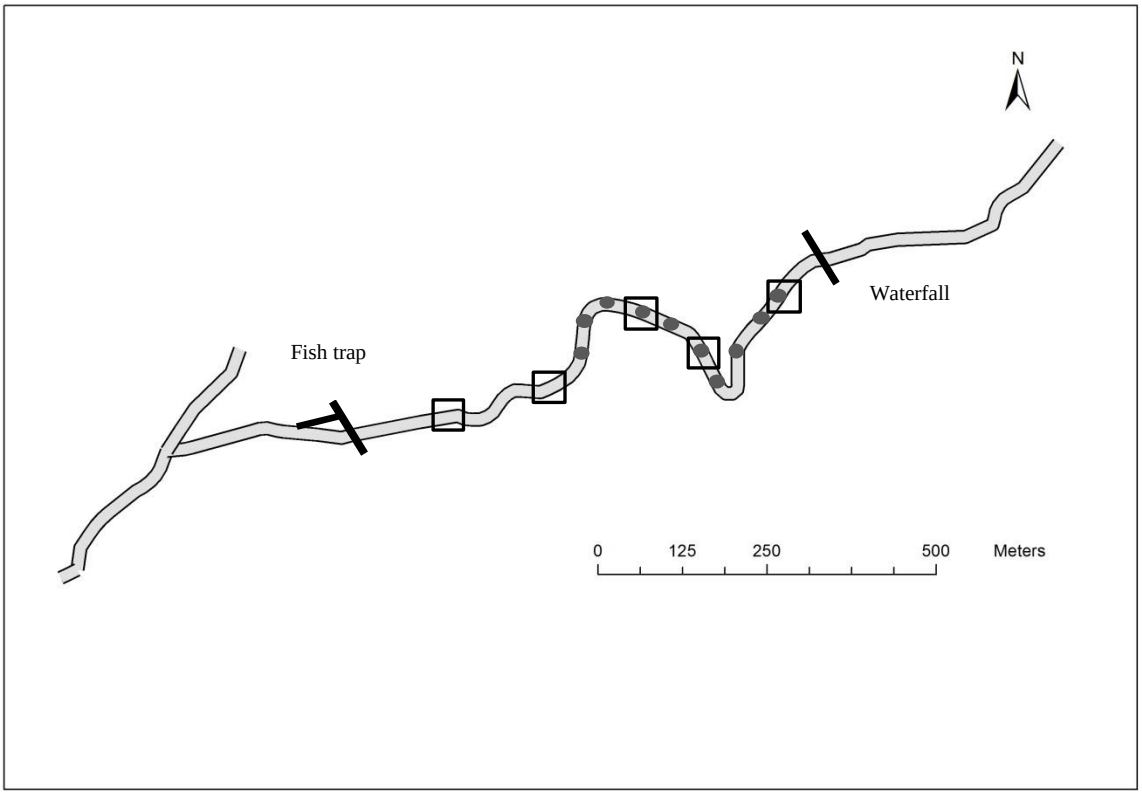

Figure 2: Experimental stretch of the Srahrevagh River, Burrishoole catchment. The location of the trapping facility and waterfall are indicated. Egg box locations are indicated by grey dots, with the location of electrofishing sites marked by black boxes

The Srahrevagh river adult trap and the screens to prevent upstream spawning were deployed in late November 2008. Natural spawning was prevented in the experimental area of the river as far as possible, and returning trapped adults were used to create three experimental groups. As parentage assignment was being used as a method of tagging, it was important to prevent natural spawning. However, the delay in setting the trap meant that some early spawners were likely to have accessed the experimental stretch and spawned naturally. This had implications for the experimental design (see Data Analysis, this section).

2.2.2 Hatchery methods

Stripping

Broodstock were collected from the Burrishoole catchment and Owenmore River during December 2008 and methods followed those of McGinnity *et al.* (2004). A total of 58 mature adults were collected, comprising 30 fish from the Burrishoole

River and 27 from the Owenmore. As in the 2004 study, an attempt was made to collect adult fish that appeared to have spent only one winter at sea (1SW). Scale samples were used to confirm age.

To produce the families, gametes were stripped from individually numbered floy-tagged mature adults into two separate labelled containers, the containers being placed on ice until fertilisation. Only 11 of the collected male Burrishoole broodstock ripened, so two fish, BM_2 and BM_3, were used to create families in both the first and second strippings (Appendix II). Fertilisation proceeded by a series reciprocal crosses, where each Burrishoole female was crossed with a Burrishoole male and an Owenmore male, and *vice versa* for each Owenmore female, to produce a total of 52 half sib families, consisting of four groups with 13 families in each group (1: Burrishoole female x Burrishoole male, 2: Burrishoole female x Owenmore male, 3: Owenmore female x Owenmore male, 4: Owenmore female x Burrishoole female).

Unfortunately due to variations in the rate at which the fish became ripe, it was not possible to synchronise the establishment of the experimental families, as had been done in the 1997 experiment and production of the families occurred over three days. The majority of the fish were stripped and the eggs fertilised on December 22nd 2008 to produce 36 half sib families and offspring groups, as listed in Table 1.

Table 1: Stripping dates and number of fish stripped on each date from each broodstock type used. See Appendix I for more detail.

Date	**Fish Stripped**			
	Females		**Males**	
22nd December 2008	9 Burrishoole	8 Owenmore	9 Burrishoole	8 Owenmore
29th December 2008	3 Burrishoole	4 Owenmore	3 Burrishoole	4 Owenmore
14th January 2009	1 Burrishoole	1 Owenmore	1 Burrishoole	1 Owenmore

A further 12 families were produced a week later on the 29th December 2008. Finally four families were established on the 14th January 2009. Of the final four families created, it was expected that only two of these would be viable, as one of the females used was not fully ripe and the eggs stripped were bloody (see Appendix I and II for broodstock details and egg survival rates by family in the hatchery). Length and weight of broodstock was recorded and scale samples were collected. Broodstock

were retained alive for disease testing and subsequently found to be disease free. Genetic samples (gill) were taken from each adult used as broodstock.

Egg Incubation

Fertilised eggs were placed in separate numbered trays in tanks in the hatchery and kept to the eyed egg stage, with dead eggs being removed on a daily basis. A record was kept of daily mortalities. Eggs were "shocked" at the eyed stage, a method used to identify and remove any eggs which were not viable. At this stage, the family groups were prepared for out-planting in the experimental river and were considered to be sufficiently robust to be handled without undue stress. Eggs were weighed (ten individual eggs) and measured (the combined length of 25 eggs in a row). Before introduction into the wild, an accurate count of the eggs in each family was obtained, then eggs were mixed and introduced into the river in artificial redds constructed according to Donaghy and Verspoor (1997). Numbers of eyed eggs planted in the experimental stretch, from each group broken down by stripping date are given in Table 2. Appendix II gives details for each family.

Table 2: Egg numbers for each family group stocked into the Srahrevagh River as eyed eggs, during three dates between February and March 2009. Stripping dates corresponded to outplanting dates, that is, first stripping eggs were outplanted first etc. BF=Burrishoole female, BM=Burrishoole Male, OF=Owenmore Female, OM=Owenmore Male.

Stripping date	Total Eyed Ova to River			
	BFxBM	BFxOM	OFxBM	OFxOM
1st stripping date 22nd December 2008	8600	8268	8165	8204
2nd stripping date 29th December 2008	3855	3809	3992	4100
3rd stripping date 14th January 2009	1285	1285	0	0
Total	13740	13362	12157	12304

Eggs were disinfected before they left the hatchery. A subsample of 25 ova from each family was retained in the hatchery. Development was checked every two days until full yolk sac absorption was reached, with mortalities and deformities recorded for each (Appendix III).

Because insufficient adult returns would have been obtained from the numbers of smolts likely to be produced in the experimental river, the marine phase of the life

cycle was examined by rearing smolts in the hatchery and releasing them to sea to complete their life cycle. A total of 13,000 eggs were held in the hatchery for on growing to the S1 smolt stage which facilitated the comparison of experimental groups in the marine environment (Table 3).

Table 3: Egg numbers for each family group retained in the hatchery as a ranching group for marine release as S1 smolts. BF=Burrishoole female, BM=Burrishoole Male, OF=Owenmore Female, OM=Owenmore Male.

Stripping date	Total Eyed Ova retained-Ranching group			
	BFxBM	BFxOM	OFxBM	OFxOM
1st stripping - 22nd Dec 2008	967	1020	2129	2544
2nd stripping - 29th Dec 2008	1252	1443	1090	234
3rd stripping- 14th Jan 2009	1047	880	0	0
Total	3266	2599	3219	2878

2.2.3 Freshwater Phase

2.2.3.1 Experimental stream populations

Eyed ova from 52 families, with the number of eggs varying among families from 430 to 1,897, were planted in the experimental stream in late February 2009. Fifty four thousand eggs were introduced into the wild, with 13,000 eggs being retained in the hatchery for rearing to smolt stage. Eggs from each family were mixed together in the hatchery before 1,000 ova were counted out into plastic wallets. Between five and six plastic wallets were placed in 11 artificial redds (Donaghy and Verspoor, 1997) along the 2km stretch of experimental stream (Figure 2).

As stated above, it was not possible in the 2008/9 to entirely exclude fish from spawning naturally in the experimental river due to late deployment of the upstream trap. In subsequent parental assignment (see Results section) it is estimated that a minimum of five females (estimated as an additional 15, 540 eggs) were introduced to the experiment in this way. These eggs were likely fertilised by multiple males (COLONY analysis suggests at least 20 individuals), likely to be predominantly local mature male parr. This group of fish are excluded from survival estimates but

included in comparisons of performance with the experimentally established groups and are designated as Burrishoole (wild) non-experimental.

2.2.3.2 Trap monitoring

The downstream component of the Srahrevagh trap began operating on the 22th April 2009 and was checked daily from that date onwards until the end of the experiment. From commencement until mid-August 2009, all 0+ fish, including an estimated ten possible brown trout, were killed and a tissue sample was preserved in 95% ethanol in individually labelled tubes. The remaining portion of the sample was frozen in an individually labelled bag. Length and weight measurements were recorded for all fish, and an individual number assigned to each sample.

After mid-August 0+ salmon and trout could be easily distinguished on sight so only 0+ salmon were killed. The trap was monitored daily for two years with all salmon being sacrificed and a tissue sample preserved in 95% ethanol, with the remainder of the sample frozen. Monitoring of the traps continued daily until 5th May 2011.

2.2.3.3 Flood event

On the night of Thursday 2nd July 2009, extremely heavy rain in the Burrishoole catchment caused extreme and catastrophic flooding in the Srahrevagh River (Figure 3), causing extensive damage to the river and in the surrounding area. Information from Met Eireann (the Irish meteorological service), based on rainfall records, classified this rainfall event as something that was likely to happen less frequently than once in every 250 years (Dunne *et al.*, 2008).

Fortunately, the fish trap on the Srahrevagh River remained operational, despite large amounts of debris, including uprooted trees and large quantities of gravel and silt, which were washed downstream and became wedged up against its screens. The trap was inundated for a period of twelve hours with the river being diverted into neighbouring fields overnight. It was possible to have the downstream portion of the trap functioning again within a number of hours, but heavy machinery was required to move large debris from the screens. No fish were captured in the trap for a period of 36 hours following its re-commissioning. A large number of 0+ fry were captured

(n=1,278) in the subsequent days. We estimate that this represents about 35% of the population extant in the river prior to the flood.

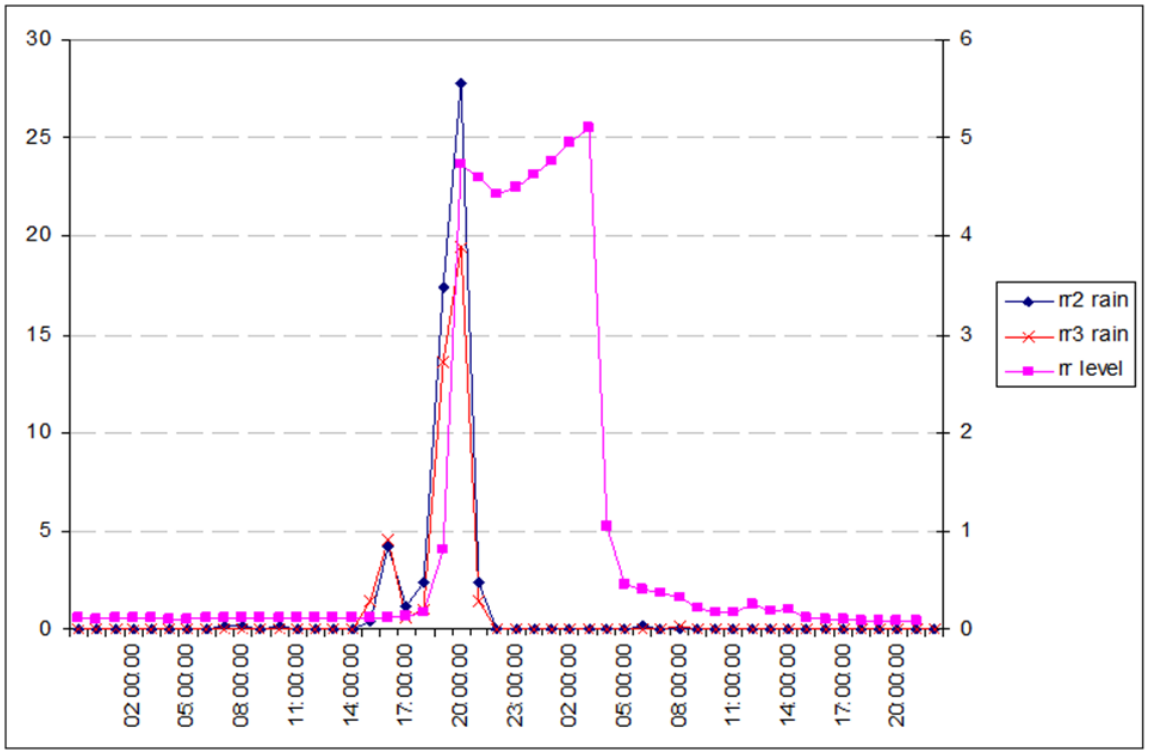

Figure 3: Rain gauge data over the 24-hour period of the extreme weather event, 2nd July, 2009, from two rain gauges (navy and red lines) and a river level recorder (pink line) located along the length of the Srahrevagh River, Burrishoole catchment. Volume recorded in cubic centimetres (courtesy of Mary Dillane, Marine Institute).

The river itself was in poor physical condition for most of the next 12-18 months of the experiment. Large amounts of gravel regularly collected at the river bend directly upstream of the trap and impeded trap operation to an extent that the gravel had to be cleared away by digger on three separate occasions. The experimental river was assessed visually at intervals post-flood and was thought to have a higher amount of exposed gravel and cobble forming the streambed. Invertebrate communities within the river were assessed on a regular basis post-flood. With regard to the Srahrevagh River, annual invertebrate surveys carried out by staff of the Marine Institute show a change in invertebrate fauna between 2010 and preceding years (de Eyto, 2011).

2.2.3.4 Juvenile stock assessment by electrofishing

In order to determine the impact of the flood and the level of migration subsequently, the experimental stretch of the Srahrevagh river was electrofished on the 9th and 10th

July, 2009, using a three pass method, to estimate the population density of 0+ salmon remaining in that portion of the river upstream of the trap. A 250 volt backpack electrofisher was used to carry out the fishing at five sections of the experimental stretch (Figure 2). Population estimates were obtained using the Zippin (1958) depletion method. All 0+ salmon collected (N = 145) during electrofishing were sampled for genetic analysis to determine the genetic makeup of salmon remaining in the experimental stretch. Juvenile population size assessments by way of electrofishing surveys were also carried out during September 2009 and August 2010. No genetic samples were collected in September 2009.

2.2.4 Marine Phase

In order to determine the relative performance of each of the experimental groups in sea, 9,500 one year-old hatchery reared smolts produced from the 13,000 eggs retained from the establishment of the experiment population in 2009, were released into the tidal Lough Furnace on the 12th April, 2010. Each smolt had its adipose fin removed in March 2010 to enable subsequent identification as a ranched fish. These were then microtagged and finally, the experimental group were freeze branded on the fish's flank near the dorsal fin before release, to differentiate them from the general ranched stock. These markings were used to identify the experimental group on their return to the adult upstream trapping facilities in the Burrishoole catchment.

Returning mature fish (n=134) from the 2010 release were recaptured at the upstream trapping facilities on the Burrishoole River during the summer and autumn of 2011 as one sea winter fish (1SW). A smaller number (19, with 11 genotyped) of two sea winter fish (2SW) returned to the traps the following year in 2012. All branded fish belonging to the experiment were culled and removed from the trap. Length, weight and sex were recorded, the microtag was removed and scale and genetic samples were collected. Analysis of the microtags confirmed the fish were part of the experimental group before samples were used for genetic analysis.

2.2.5 Molecular analysis

A total of 1,979 juvenile salmon were collected by means of the downstream trap or electrofishing between 22nd April, 2009, and 5th May, 2011. Genotyping is expensive and within the constraints of the available resources a sub-set of 998 samples (approximately half of the fish) were genetically screened to allow parentage assignment. Table 4 shows the number of fish collected and the number analysed for parentage assignment. To confirm the proportions of the four population groups within the ranch population relative to their composition at the egg stage, a random sample of 400 ranched smolts were sampled and genotyped. All adult returns (n=144) captured in the Burrishoole river traps were genotyped. A breakdown of samples collected and analysed is given in Table 4.

Table 4: Breakdown of samples-time of collection, total number of samples collected and size of subsamples subsequently analysed genetically for freshwater and marine experiments.

Sample type	Time samples collected	Life stage	Total	Subset analysed
Pre flood	April - early July 2009	0+	412	298
Flood	July 2009	0+	1278	442
Electrofishing	July 2009	0+	176	145
Wild Smolts	February - April 2011	wild smolts/p males	113	113
Freshwater Total			**1979**	**998**
Ranched Smolts	April 2010	S1 smolts	9115	400
Adults	Summer 2011/ Summer 2012	adult returns	144	144
Marine Total				**554**

The first 1,000 samples (freshwater portion) for this project were extracted using a salt-based method, and run on a LI-COR 4200 automated DNA sequencer. The remaining samples used a plate based extraction method and were analysed on an ABI 3730XL Analyser (96 capillary, ABI Applied Biosystems, Ltd.).

DNA for the initial 1,000 samples was extracted from tissues by using the Puregene DNA extraction procedure (Qiagen Ltd), a salting-out method which gives high molecular weight, archival quality DNA. Quantification of extracted DNA was carried out by spectrophotometry, using a Nanodrop ND-1000, and quality was

assessed by running a subsample from each group of 96 individual samples on a 1.5% Agarose gel. DNA was diluted to between 20-40µg. Individuals were screened for variation at 10 microsatellite loci: *MHC 1, MHC 2, Sp2210, Sp2216, Ssa171, Sp3016, Ssa197, SSaD170, Ssa85, Ssa197* and *SsaD71*. Amplifications were carried out in 10µl volumes, which included 1µL of extracted DNA 0.25 mM dNTPs, 0.5 U *Taq* DNA polymerase, 2µl of 5x buffer, 0.5mM $MgCl_2$ and 1µM each of forward and reverse primers. The PCR reactions were carried out on a Techne TC-Plus thermocycler and followed an initial denaturation period of 95°C for 3 minutes, 30 cycles of 95°C for 30 seconds, annealing at 56°C for 30 seconds, and extension at 72°C for 30 seconds. Alleles were resolved using 6% denatured polyacrylamide gels on a LI-COR 4200 automated DNA sequencer. The sizes and weights of alleles were determined using a molecular weight marker (LI-COR) and allele cocktail of most common genotypes to ensure consistent scoring. An experienced second reader checked a subset of genotypes to ensure consistency of scoring.

For the remaining samples, which were ran on the ABI system in the Molecular Ecology Laboratory in Queens's University Belfast, DNA was extracted from tissue samples by using the Wizard SV 96 Genomic DNA Purification System (Promega Ltd), which gives high molecular weight, archival quality DNA. Quantification of extracted DNA was carried out by spectrophotometry, using a Nanodrop ND-1000, and quality was assessed by running a subsample from each group of 96 individual samples on a 1.5% Agarose gel. DNA was diluted to between 4-8 µg and these diluted samples were plated out into 96 well plates with two control samples and a water blank on each plate.

Amplification reactions were carried out with a total reaction volume of 3.5µl for eight neutral microsatellite loci (excluding the MHC linked loci) using five multiplex panels. The PCR reaction consisted of 1 µl DNA extract, forward and reverse primers at a concentration of 20 pM/µl and PPP Master Mix (2x concentrated, Top Bio Ltd.) made up of 150 mM Tris-HCl, pH 8.8 (at 25° C), 40 mM $(NH_4)_2SO_4$, 0.02% Tween 20, 5 mM $MgCl_2$, 400 µM dATP, 400 µM dCTP, 400 µM dGTP, 400 µM dTTP and 100 U/ml of *Taq* DNA polymerase. The recipes for the multiplex panels are provided in Appendix IV. PCR reactions were carried out using a Techne TC-Plus thermocycler. The PCR cycle followed an initial denaturation period of

95°C for 3 minutes, 30 cycles of 95°C for 30 seconds, annealing at 56°C for 30 seconds, and extension at 72°C for 30 seconds. Diluted, prepared samples of PCR product were shipped from the Population Genetics Laboratory in UCC to Queens University, Belfast (QUB), where they were denatured and alleles were resolved on an ABI 3730XL Analyser (96 capillary, ABI Applied Biosystems, Ltd.). Fragment length was determined using GeneScan 500-LIZ size standard and alleles were scored using Genemarker v1.6 (Applied Biosystems). The accuracy of scored alleles was checked by a second reader scoring a random subsample of individuals. The dataset contained ten loci: *MHC 1, MHC 2, Sp2210, Sp2216, Ssa171, Sp3016, Ssa197, SSaD170, SSOL85, Ssa197* and *SsaD71*, but the MHC loci were excluded from the assignment analysis. We switched to the QUB ABI for genotyping midway through the project in order to speed up the acquisition of genetic information.

A comparison of scoring of the same alleles on the two systems using a control panel of genotypes (carried out by Dr. Eileen Dillane, UCC) showed some consistent differences between scoring for some alleles using the two systems. Results were standardised by correcting alleles from the ABI system to match those sizes recorded for genotypes analysed using the LI-COR system.

2.2.6 Data Analysis

2.2.6.1. Parentage Assignment-all parts of life cycle

Parental assignment analysis was carried out using PASOS (Duchense *et al.,* 2005) and Colony (Jones and Wang, 2009). Eight neutral microsatellite markers (excluding MHC linked loci) were used for parentage assignment. A total of 1,519 offspring were analysed, based on genotypes from 52 possible parents. There was a lower than expected assignment rate of 83% or 1,254. This assignment rate allowed for no genotyping errors (with careful quality control of the dataset). Allowances for a 5% rate of error in the dataset suggested several crosses, which were impossible, so only results produced from analyses allowing for no rate of error were accepted. It would appear that salmon spawned in the experimental river in addition to those that were artificially introduced. Evidence for natural spawning, i.e. the existence of the offspring fish not used to establish the experimental population, contributing

individuals to the trap and electrofishing samples is supported by two observations: 1. Analysis carried out using PASOS and Colony software suggested multiple possible parents for the remaining unassigned component of the dataset (17% of total dataset, 265 offspring), and 2. In contrast to the freshwater situation, there were only three unassigned offspring out of a total sample of 524 offspring analysed for parentage assignment, in the hatchery sample (0.6% of marine sample was unassigned).

The breakdown of unknown parents seems to follow patterns that would reflect typical mating behaviour in the wild. Several putative parents were suggested by the analysis, with three "female" and two "male" parents responsible for the parentage of 79 unassigned offspring (30% of unknown freshwater component). Five suggested "female" genotypes account for 206 unassigned offspring overall, with a mixture of up to twenty suggested male parental genotypes possible. This number probably includes a number of false parental genotypes due to undetected scoring error in the offspring, as an error in one allele for one offspring fish would result in a new parental genotype being produced. While extensive data quality control and second scoring was carried out on the data it was more difficult to detect small errors in unassigned offspring as there were no defined parental genotypes for comparison, so a certain level of error cannot be discounted. However, these results confirm wild spawning of mature adult fish that managed to migrate upstream in the experimental stretch of the Srahrevagh, plus the very likely input of mature male parr.

2.2.6.2 Genetic Analysis

A total of 1,552 individual fish were successfully genotyped. Scored alleles were checked for genotyping errors using Microsat Toolkit (Parks, 2001). Allele number and allelic richness were calculated using the computer programme FSTAT v2.9.3 (Goudet, 2001). The percentage number of alleles observed in each sample, in relation to the total number of alleles observed among all samples was calculated. Observed (H_o) and expected (H_e) heterozygosities among populations were estimated using the programme GENEPOP v4.2 (Raymond and Rousset, 1995). Deviations from Hardy-Weinberg Equilibrium were estimated using MCMC (Markov Chain Monte Carlo) with 10,000 dememorisation steps, 1,000 batches and 10,000 iterations per batch using GENEPOP. Genotypic linkage disequilibrium within populations

was tested for using GENEPOP to ascertain if genotypes at one locus were independent from the genotypes at another locus. Estimates of genetic differentiation were generated using unbiased F_{ST} (Weir and Cockerham, 1984). These values were calculated by GENEPOP so as to be comparable with estimates from previous studies.

2.2.6.3 Statistical Analysis

As relative sizes of groups in some samples are determined by both migration and survival, this is referred to in the text as "representation". Testing for significant differences between survival and representation of the different non-native and hybrid groups relative to the native group was carried out with G tests, using a Williams's correction (Sokal and Rohlf, 1995, McGinnity *et al.*, 2004) and were expressed relative to native (local) value of 1.

The distribution of length, weight, condition factor, fecundity and time of return data was found to approach normality and to be equally variant in the majority of cases, so was analysed using a one-way ANOVA to test for differences between groups. Overall significant differences were then assessed using Tukey's pairwise comparison tests. Where data was not normally distributed or had unequal variances, a non-parametric Kruskall-Wallis test was used to determine significant differences.

Heterosis (or hybrid vigour) refers to the phenomenon that first generation offspring of diverse species or populations exhibit greater trait performance either in terms of biomass, development or fertility than the better of the two parents (Gjedrem, 2005). The degree of heterosis was calculated as the difference between the mean performance of the hybrid groups (BFxOM and OFxBM), both combined and independently, and the mean performance of their pure parental groups (BFxBM and OFxOM). This was expressed as a percentage of the mean performance of the parental groups and is referred to as mid-point heterosis (MPH) after Bourden (1997). MPH was calculated for survival, maturation and dispersal rates. Significance between groups was tested using one-way ANOVA to test for differences and overall differences were then analysed using Tukey's pairwise comparison tests.

2.3 Results

2.3.1 Genetic Variation

A total of 52 adult broodstock *Salmo salar* which were successfully used (from 58 collected) to create 52 half-sib families from two catchments, were genotyped across eight neutral loci. No problems with consistent genotyping errors were found with Micro-Checker. Genetic differentiation analysis show the Burrishoole and Owenmore broodstock to be significantly (p=0.027) differentiated with an overall Pairwise F_{ST} value of 0.0361 with individual loci values ranging from 0.0059 to 0.1022. This overall value is consistent with other studies (NGSI report, 2008, de Eyto *et al.*, 2011), which found evidence of significant levels of genetic differentiation (F_{ST} of 0.0316) between Owenmore and Burrishoole populations. Summary genetic data for parents are given in Appendix V. The number of samples analysed at each locus are provided, along with the number of alleles and allele frequency for each locus. Allelic richness (A_R) and expected heterozygosity (H_E) were used to indicate within population genetic variability. Observed (H_O) and expected heterozygosity (H_E) are presented, and significance values of HWE were corrected using a Bonferroni correction (0.05/8=0.00625). No consistent deviations from HWE (across samples) after a standard Bonferroni correction were found for any of the loci examined (Appendix V).

2.3.2 Fertilization to eyed egg stage and hatchery stage

2.3.2.1 Broodstock details

Mature adult Atlantic salmon were collected from the Burrishoole (n=31) and Owenmore (n=27) catchment during November and December 2008. Thirteen males and females from each catchment were used as broodstock to establish the experimental population (details given in Appendix II). Females from both catchments were not significantly different from each other in terms of length or average egg size, which was measured in terms of volume of 200 eggs per female (Table 5). Female salmon from the Owenmore did produce a slightly higher than average number of eggs per fish when compared to Burrishoole females of the same

size, although this was not significant (Table 5). Fish were not weighed during the stripping process so it was not possible to compare condition factor.

Table 5: Mean values and standard errors (S.E.) for length, number of egg stripped and egg volume for all broodstock (n=52) from the Burrishoole and Owenmore adult salmon used in the experiment to create the 52 experimental families.

Group	Age	n	Average Length (cm)		n(eggs) stripped		Volume of 200 eggs (ml)		Average Eggs/kg	
			Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Burr Fem	All	13	65.2	2.3	3185	390	45.92	1.79	1345	120
	1SW	8	61.9	2.8	2898	363	43.31	1.3	1407	439
	2SW	5	70.48	2.7	3644	855	50.1	3.7	1245	225
Owen Fem	All	13	65.4	1.4	4016	390	47.32	1.61	1637	71.1
	1SW	9	62.69	0.65	3771	183	44.28	1.3	1712	64
	2SW	4	71.63	2.1	4566	950	54.25	0.25	1470	166
Burr Male	1SW	11	59.6	1.06						
	2SW	0	-	-						
Owen Male	All	13	68.15	1.99						
	1SW	10	64.27	1.1						
	2SW	3	76.9	2.6						

It was possible to model weight values per kg based on length data obtained, this allowed us to use the standard comparison of eggs per kg for female salmon. T-tests showed no significant differences between the number of eggs per kg for either age classes or between Burrishoole and Owenmore female broodstock (Table 6). Owenmore male salmon were found to be significantly longer than Burrishoole males (Table 6). Two-sea-winter (2SW) Owenmore female salmon were longer and had larger egg sizes than 1SW females from the same site (Table 6). There were no significant differences found between 2SW and 1SW females from the Burrishoole when looking at the same parameters (Table 6). All Burrishoole male broodstock collected were 1SW. Two–sea-winter (2SW) Owenmore males were found to be significantly larger (Table 6) than 1SW male fish from the Owenmore. Full details of broodstock used in creating families for this experiment are given in Appendix I. Details of stripping events and creation of families and groups, including egg counts and sizes for each female are given in Appendix II.

Table 6: Results for two-sample t-tests of average values for broodstock collected for the experiment. Significant values are given in bold. BF=Burrishoole Female, BM=Burrishoole Male, OF=Owenmore Female, OM=Owenmore Male.

Group for t-test	n	Length (cm)	n(eggs) stripped per female	Volume of 200 eggs (ml)	Average Eggs/kg
BF vs. OF	**26**	df= 19, p=0.93	df=22, p=0.1	df=23, p=0.56	df=19, p=0.06
BM vs. OM	**24**	**df=18, p=<0.001***	-	-	-
BF 1SW vs. 2SW	**13**	df=10, p=0.054	df=5, p=0.459	df=4, p=0.154	df=7, p=0.573
OF 1SW vs. 2SW	**13**	**df=3, p=0.008***	df=8, p=0.472	**df=3, p=0.008***	df=3, p=0.267
OM 1SW vs. 2SW	**13**	**df=4, p=0.011***	-	-	-

2.3.2.2 Green egg to eyed egg survival in the hatchery

Full counts of eggs in each family and survival rates recorded are given in Appendix III. There were no significant differences in terms of number of eggs that died between groups, either at the green egg (ANOVA, p=0.218) or eyed egg stages (ANOVA, p=0.658). Egg sizes were also found not to differ significantly between groups (ANOVA, p=0.869).

Table 7: Mean numbers and standard errors for green eggs and eyed eggs, and for group survival (%) for all families created on each of the three stripping dates.

Sample	Stripping	N	Mean	SE
Green eggs	1	34	1627.6	98.4
	2	14	1815.1	82.7
	3	4	2712	373
Eyed eggs	1	34	1354.1	93.7
	2	14	1492	112
	3	4	2497	344
% Family Survival	1	34	83.5	2.04
	2	14	81.43	3.98
	3	4	92.08	0.98

Examining the numbers of green eggs produced and surviving numbers of eyed eggs for the two main stripping dates (Stripping One and two, Table 7, Figure 4), there were no significant differences in egg survival at either stage, 83.5% and 81.43% respectively (t-test, df=42, p= 0.152 and df=31, p=0.353 respectively, Table 7).

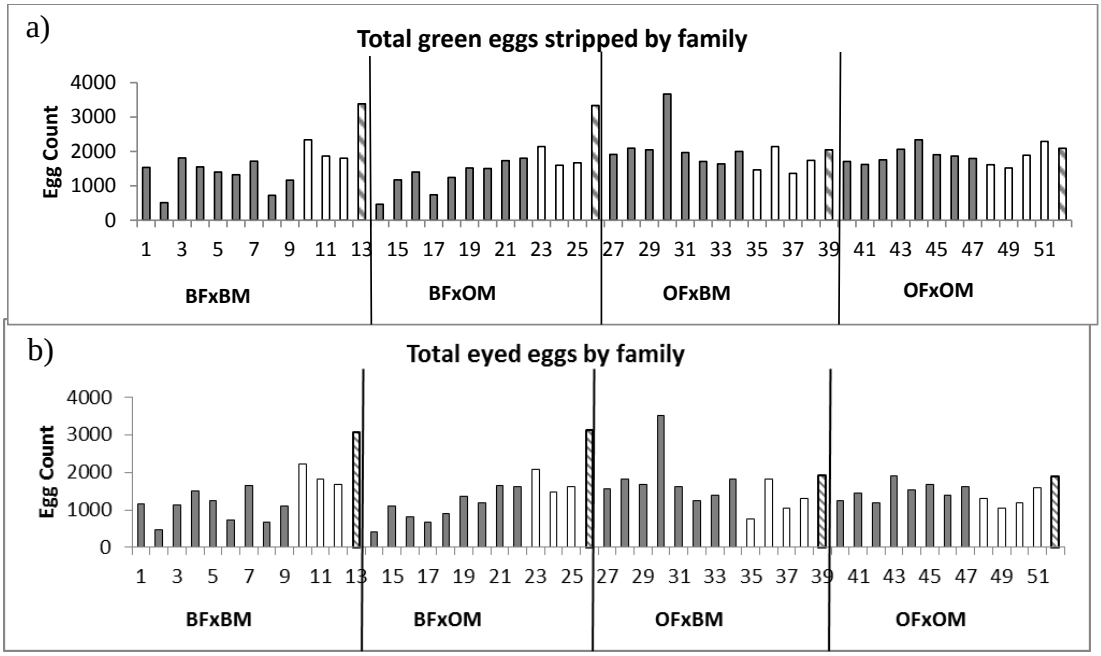

Figure 4: Counts of each family and group for a) green eggs and b) eyed eggs in the hatchery. Solid bars indicate the first stripping date, empty bars indicate the second stripping date, and the third stripping date is shown by hatched bars. BF=Burrishoole Female, BM=Burrishoole Male, OF=Owenmore Female, OM=Owenmore Male.

The survival of the experimental population by group and by family is given in Table 1 (means and standard errors by group and family) of Appendix II. The green egg to eyed egg survival for the experimental population overall was 83.96%. Green egg survival varied significantly among the four groups (Kruskall Wallis: p=0.013) and was poorest for the eggs produced by Owenmore females, whether crossed with an Owenmore male (77.7%, S.E. =±3.03) or Burrishoole male (81.5%, S.E. =±3.18) compared to 87.2% (S.E. =±3.8) survival at this stage for the pure Burrishoole cross and 87.9% (S.E. =±3.26) for the Burrishoole female by Owenmore male cross. Within the four groups, the greatest variability in family mortality was observed in the local Burrishoole population (2.5% to 44.3% mortality) and was found to be lowest in the pure Owenmore cross (8% to 38% mortality). No families failed to survive from the green egg to eyed stage, as can be seen in Figure 4.

No significant differences were found in overall rates of family survival when comparing between the two main stripping dates on the 22nd and 29th December 2008, when 48 out of the total 52 family groups were created (t-test, df=20, p=0.648 Appendix II, Table 1).

2.3.2.3 Survival of Families/Groups post eyed egg stage in the hatchery

After parentage assignment of collected offspring, both in the trap and by electrofishing, four half-sib family crosses out of the original 52 families established returned no offspring for any of the life stages observed. These four families were the four families created during the third stripping, BF_13xBM_10, BF_13xOM_14, OF_13xBM_10 and OF_13xOM_14. It is possible that these groups failed to hatch successfully in the common garden experiment in the wild. Mortality to hatching and yolk absorption of each family was monitored in the hatchery by retaining a small subsample of 25 eggs (for details, see Appendix III). The samples retained for OF_13xBM_10, and for OF_13xOM_14 were the only example recorded of poor mortality, with 14 alevins surviving to the yolk absorption stage out of an original sample of twenty five eggs for the first group, with only one surviving alevin in the second group. The two family groups using BF_13 showed no recorded mortalities to the alevin stage in the hatchery test. All other family groups showed no unusual rates of mortality or deformity in the hatchery subsamples.

2.3.3 Juveniles in freshwater

Details of the numbers of recovered fish and samples identified to group by parentage assignment for all parts of the freshwater phase of the experiment are presented in Table 8. G test results for observed frequency differences relative to the native Burrishoole group are given in Table 9. Unassigned fish are also included. These fry are considered the result of natural spawning within the experimental stretch. Abundance of a group in a sample is determined by both mortality and emigration from the experimental river and is therefore denoted as ‘representation’.

For the first ten weeks of trap operation, a total of 412 0+ salmon were recorded in the downstream trap on the Srahrevagh River from April 22nd 2009 up to and including the 2nd July 2009.

Table 8: Counts of offspring identified to group level by parentage assignment. Unassigned offspring refers to those fish assumed to be sourced from natural spawning within the experimental stretch of river. BF=Burrishoole Female, BM=Burrishoole Male, OF=Owenmore Female, OM=Owenmore Male.

Samples	Total Collected	Total Assigned	BF X BM	BF X OM	OF X BM	OF X OM	Unassigned
2009							
Pre-flood Fry	412	297	28	45	44	83	97
Flood migrating fry	1278	442	72	94	101	109	66
Post-flood electrofishing	145	145	22	33	29	22	39
2011							
Precocious males	29	29	6	7	3	5	8
Wild smolts	33	33	8	4	6	11	4
Combined smolts and prec. males	62	62	14	11	9	16	12

The extreme weather event recorded on the night of 2nd July 2009 resulted in high migration of 0+ fry through the trap in the days immediately afterwards. A total of 1,278 0+ salmon (an estimated 35% of the experimental population in the river up to that time, as stated above), were caught in the downstream trap in the five days following the flood. It had been planned to carry out extensive electrofishing of the experimental stretch during August/September of 2009 to estimate the survival of the four groups to the 0+ stage, to correspond with the 0+ electrofished sample from McGinnity *et al.* (2004), collected in late August of the first year of that experiment. The flood event of the 2nd July provided a large sample in the downstream trap and it was decided that the flood sample, assuming no bias in the groups or families displaced, would fulfil the same purpose as the planned electrofishing. No 0+ trout were recorded migrating through the trap at this time.

Due to concern over the possible low density of the remaining 0+ salmon fry after such an unusual and extreme weather event, the experimental river was electrofished at five sites to estimate remaining population density of 0+ salmon fry. One hundred and forty five 0+ fry were collected for genetic analysis (Table 8). From the electrofishing survey of the 9th and 10th July it was estimated there were 2,543 0+ salmon remaining in the experimental stretch.

Each section of the freshwater portion of the experiment showed representation of juvenile salmon that we believe were the offspring of naturally spawning salmon.

Colony analysis suggests five main maternal contributors to the genotypes of these suspected "wild" offspring. From this we estimated the number of ova produced by taking the estimated fecundity values for the Burrishoole catchment for 2009. Average fecundity for mature female salmon collected from the experimental stretch as broodstock in this experiment was estimated at 3108 eggs per fish (pers comm. E de Eyto, Marine Institute). We therefore estimated that 15,540 eggs were deposited through natural spawning. This is an approximation, so where presented these data are shown as striped or in hatched lines to differentiate them from the fish that were introduced as part of the experimental population.

2.3.3.1 Survival and Representation in freshwater

Figure 5 gives the representation of the four experimental groups, either as migrants through the trap (pre-flood and flood samples, migrating smolts and precocious males) or as present in the river (electrofishing sample) for the freshwater phase of the experiment. Adult returns to the upstream traps at the freshwater/marine interface of the Burrishoole catchment are also presented. All results are described in the following sections. Table 9 shows the significance values for differences between proportions of collected offspring from each group of the freshwater phase of the experiment.

Table 9: Significance values for G-tests performed on collected offspring from each group in the freshwater phase of the experiment. Overall values for tests between the four groups are given, along with values for the two hybrid (BF X OM, OF X BM) and the foreign group (OF X OM) compared to the local group (BF X BM).

Samples	G tests vs. BF X BM	BF X OM	OF X BM	OF X OM	Overall G tests (all four groups)
0+					
Pre flood		p=0.061	**p=0.046***	**p<0.001***	**p<0.001***
Flood		p=0.07	**p=0.02***	**p=0.004***	**p=0.021***
Post flood		p=0.416	p=0.35	p=0.97	p=0.319
1+					
Precocious Males		p=0.96	p=0.36	p=0.82	p=0.636
Smolts		p=0.41	p=0.82	p=0.6	p=0.293

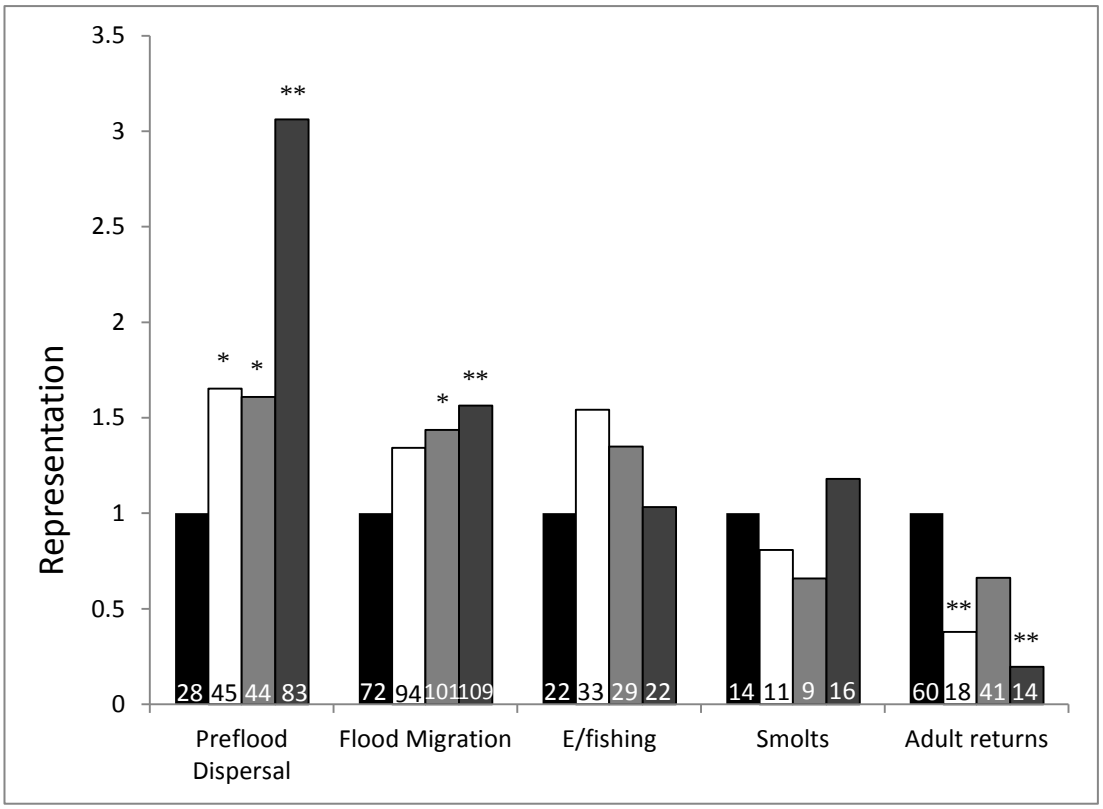

Figure 5: Representation of each group at each sampling stage for the freshwater phase of the experiment. Counts of each group are given in the bars. BFxBM = black bars, BFxOM= white bars, OFxBM=light grey bars, OFxOM= dark grey bars. BF=Burrishoole Female, BM=Burrishoole Male, OF=Owenmore Female, OM=Owenmore Male. Significant differences from BFxBM are indicated by * where $p<0.05$ and by ** where $p<0.01$.

2.3.3.2 Early 0+ migrants-Preflood

During the period from the start of trap operation on the 22nd April 2009 until the 2nd of July 2009, 418 fry migrated in to the downstream trap. When the parentage of fish caught was determined, there was significantly higher proportion (G-test, $p < 0.001$, Table 9) of the Owenmore group represented in the trap sample relative to the Burrishoole group. Three times more "pure" Owenmore fish (OFxOM cross, 86 0+ fish) migrated when compared to offspring with Burrishoole parents (BFxBM cross, 28 0+ fish). Hybrids between the two groups fell between these values, with 1.6 times more fish from both of the hybrid groups migrating through the downstream trap than native fish (Figure 5, 45 and 44 0+ fish respectively). There were also significantly more hybrid fish represented (Table 9) than native fish. No significant differences in juvenile dispersal were found between the hybrid groups.

Table 10: Significance values and groups significantly different to the local group (BF X BM) for length (cm), weight (g) and condition factor for all sampling stages in the freshwater phase of the experiment.

Sample	Sample size	Significance values			Groups sig. different to BF X BM		
		Length (cm)	Weight (g)	K factor	Length (cm)	Weight (g)	K factor
Preflood	297	p=0.590	p=0.124	p=0.082	-	-	-
Flood	442	p=0.363	p=0.087	**p=0.03***	-	-	OF X OM
Electrofishing	145	p=0.884	p=0.612	p=0.062	-	-	-
Precocious Males	29	p=0.53	p=0.303	p=0.636	-	-	-
Smolts	33	p=0.248	p=0.15	p=0.996	-	-	-

Length, weight and condition factor show no significant differences between the different groups caught in the trap before the flood (Table 10 and further details in Appendix VI). The "wild" group, i.e. the offspring of the naturally spawning fish, were also represented at a significantly greater proportion in the trap when compared to the native Burrishoole group.

2.3.3.3 Main 0+ parr sample

Based on the fish caught in the trap during flood (1,278) combined with the estimated number of juveniles left in the river after the flood (2,417, based on electrofishing data, Appendix VII), it was estimated that the mortality rate from egg to 0+ salmon in the river of the experimental population was 94.67%. The parentage analysis of the fish caught in the trap during the flood shows significant differences in representation among the groups in the fish caught in the trap immediately after the flood (Table 9), with the offspring of OFxOM (foreign, 109 fish), OFxBM hybrid (101 fish) and BFxOM hybrid (94 fish) being 50%, 40% and 31% over-represented respectively relative to the BFxBM (local, 72 fish) group (Figure 5).

Ten days after the flood, on the 12 July, a sample of 145 fish was collected by electro-fishing from the experimental river for parentage assignment. In this sample, there were equal numbers of BFxBM and OFxOM fish (22 fish in each case) with over-representation of the two hybrid groups, BFxOM (50%, 33 fish) and OFxBM (32%, 29 fish), although these were not significantly different from the native group (Table 9). The addition of the estimated number of fish remaining in the river (2,272), reallocated to the four groups on the basis of the proportions suggested by

the genetic analysis of the electro-fished sample and the fish displaced in the flood on the 2nd July, gives a total of 3,695 0+ salmon and a survival of egg to 0+ parr of 5.33%. This gives an approximate idea of the composition of the population immediately prior to the flood, with estimated over-representation of the two hybrid groups at 47% (BFxOM) and 43% (OFxBM) respectively and the OFxOM group at 23% of local fish (BFxBM).

There were no significant size differences among the experimental groups in the fish that were displaced by the flood, nor were there any differences in size among the groups not displaced by the flood (Appendix VI). There was, however, a significant difference in the size of the fish caught in the trap and the fish remaining in the river with the resident fish being 7% longer (t-test: p.0001) and 16% heavier (t-test: p.0001). Also, the displaced fish had a higher condition factor of 1.06 as compared to 1.00 for the residents (t-test: p.0001, Table 11).

Table 11: Mean and standard error (S.E.) values for length (cm), weight (g) and condition factor for the sample of fish collected from the flood event and those collected in the post-flood electrofishing.

Sample	N	Length (cm)		Weight (g)		Condition factor	
		Mean	S.E.	Mean	S.E.	Mean	S.E.
Flood	437	4.025	0.017	0.706	0.01	1.057	0.008
E/fishing	145	4.337	0.031	0.83	0.019	0.997	0.009

2.3.3.4 Wild naturally spawned up to 0+ stage

More than three times the number of the offspring of Burrishoole fish that spawned naturally in the Srahrevagh River were captured in the trap prior to the flood when compared to experimentally-introduced fish of Burrishoole parents, showing a significant difference in representation between groups (G-test, $p<0.001$, 97 wild fish, 28 from the BFxBM group). Their estimated survival compared to experimentally-introduced Burrishoole group was greater by some 29% i.e. by combining fish caught in the trap during the flood and estimated numbers in the river after the flood. In the actual flood itself the wild group were 8% under-represented compared to the Burrishoole experimental group and were 65% over-represented in the sample collected in the river after the flood, although this result was not significant (Figure 6, G-test, $p=0.268$ and $p=0.108$ respectively). In terms of length and weight, the naturally spawned Burrishoole origin fish caught in the trap

immediately after the flood showed no significant differences to their experimentally produced con-specifics. This is also the case for the fish retained in the river after the flood.

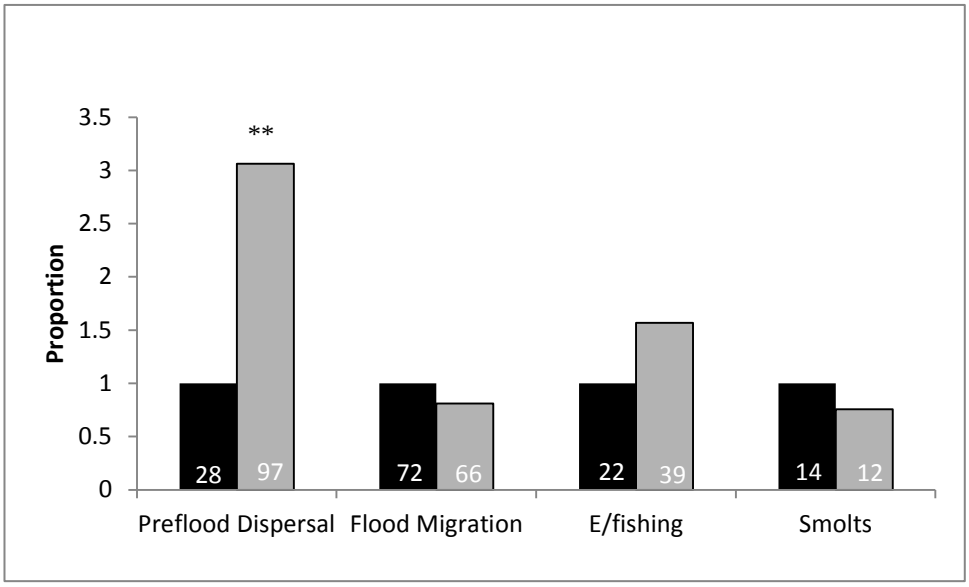

Figure 6: Representation of BFxBM (Black bars) and Wild (naturally spawned, grey bars) at each sampling stage for the freshwater phase of the experiment. Counts of each group are given in the bars. ** indicates a significant difference in representation where $p<0.001$.

2.3.3.5 Smolt stage

The overall survival of the experimental stream population from eyed egg to smolt was 0.09%. The survival of the experimental population for intermediate stages between 0+ salmon and smolts, namely over-winter survival of 0+ parr in September 2009 to the 1+ parr stage in August 2010 was 56.5%. The survival rate subsequent the second winter from August 2010 to the smolt migration in spring 2011, was estimated at 7.0%.

The relative representation of the four groups in the out-migrating smolts did not vary significantly (Table 9), which may be due to the small sample sizes. However, representation values relative to the experimental local BFxBM group (14 fish) were substantial for the two hybrid groups BFxOM and OFxBM (20% and 34% under-represented respectively, 11 and nine fish, Figure 5), with the OFxOM group being 18% over-represented (16 fish, Figure 5).

The egg to smolt survival values were similar across the four groups, ranging from 7% to 12%, with no significant differences found in survival rates across the groups. There were no significant differences in size among the experimental groups at the smolt stage (Figure 5, Table 9).

2.3.3.6 Wild naturally spawned from 0+ to smolt

In terms of representation at the smolt output stage the offspring of the Burrishoole naturally spawned salmon are under-represented by 20% relative to the Burrishoole experimentally introduced offspring (12 fish), a difference which was not significant (BFxBM, Figure 6, G-test, p=0.621). Again, there was no significant difference in size between the two Burrishoole groups, irrespective of origin. Like the two hybrid groups, the naturally spawned fish survived better in the summer immediately after emergence and poorer subsequent to the flood compared to the experimentally introduced Burrishoole fish.

2.3.3.7 Freshwater survival and representation by spawning date

As described in the Methods section, broodstock ripened on various dates, leading to three distinct stripping dates and three different dates for the introduction of the eyed eggs to the experimental river. No significant differences were found when comparing green and eyed egg survival between the two main stripping dates (earlier, this section) in the hatchery. Representation data was analysed by the first two stripping dates to see if there was a significant difference in survival between the two strippings. The impact of the third stripping was thought to be minimal as it was not present in any of the freshwater stages, with the exception of the flood sample which had four individuals from a total of 442 sampled from the third stripping (Figure 7). Due to its small size and poor survival, the third stripping was excluded from further analyses.

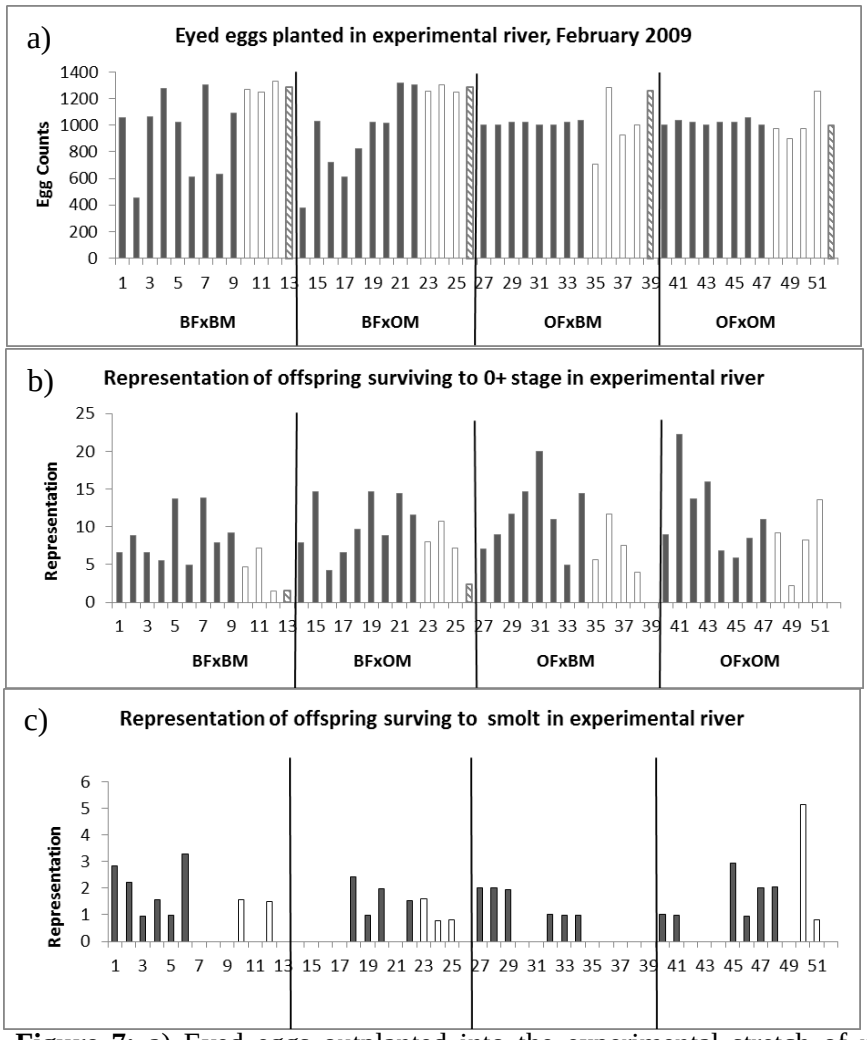

Figure 7: a) Eyed eggs outplanted into the experimental stretch of river on three dates, represented by group. b) Surviving offspring to 0+, corrected with egg numbers, shown by stripping date and group. c) Surviving offspring to smolt (corrected with egg numbers and standardised to 1000 eggs, shown by stripping date and group. Stripping one (grey bars, 34 families) were outplanted on the 22nd February 2009, second stripping (white bars, 14 families) outplanted on the 29th February, and the third stripping outplanted on March 2009, (four families). BF=Burrishoole Female, BM=Burrishoole Male, OF=Owenmore Female, OM=Owenmore Male.

It was found there were no major significant differences in results for salmon survival/representation at any stage of the freshwater phase examined, with the majority of results being very similar to combined analyses, showing the same pattern of representation in nearly every case (Table 12). Table 12 shows the results (p values) of all tests carried out. These results allowed for the acceptance of analyses of group representation and length, weight and condition factor analyses based on collated data between the stripping dates.

Table 12: Significance levels of means for groups of representation, length, weight and condition factor in the experimental river, split by stripping date.

Sample	N	Representation	Length (cm)	Weight (g)	K factor
Preflood					
Combined	200	BFxBM<BFxOM<OFxBM<OFxOM	p=0.590	p=0.124	p=0.082
1st strip	151	pattern the same as combined	p=0.192	p=0.221	p=0.536
2nd strip	49	pattern the same as combined	p=0.919	p=0.761	p=0.141
Flood					
Combined	372	BFxBM<BFxOM<OFxBM<OFxOM	p=0.363	p=0.087	**p=0.03***
1st strip	282	pattern the same as combined	p=0.146	p=0.275	**p=0.002**
2nd strip	90	pattern the same as combined	p=0.192	p=0.093	p=0.095
E/fishing					
Combined	106	(BFxBM=OFxOM)<OFxBM<BFxOM	p=0.884	p=0.612	p=0.062
1st strip	84	pattern the same as combined	p=0.859	p=0.915	p=0.627
2nd strip	22	pattern the same as combined	p=0.450	p=0.645	p=0.758
Smolts					
Combined	50	BFxBM<OFxOM>BFxOM>OFxBM	p=0.141	p=0.227	p=0.816
1st strip	35	pattern the same as combined	p=0.093	p=0.462	p=0.471
2nd strip	15	pattern the same as combined	p=0.672	p=0.813	p=0.261

2.3.4 Marine Survival

In the hatchery 13,000 fertilised ova were retained as a ranching group and reared as one year old smolts. A total of 9,115 S1 smolts were successfully released to sea in May 2010, giving an overall hatchery survival rate to S1 of 71%. Since the experimental population was managed as a single group within the hatchery it was not possible to know exactly the group and family composition of the ranched smolts as released in April 2010. Two estimates (Table 13) of the likely make up of the release group are provided. The first assumes that mortality within the hatchery was constant among the groups and families and hence the initial starting egg numbers are used to estimate survival values. The second estimate is based on a genetic analysis of a sample of the outgoing smolts (n=381). Although both estimates appeared to vary slightly, this was not significant (G test: $p>0.06$). Therefore, the estimates are sufficiently similar so as to not impact on our confidence of our estimates of group marine survival. Figure 8 shows breakdown of hatchery egg numbers for each family at each stripping and numbers of marine returns by family and stripping date.

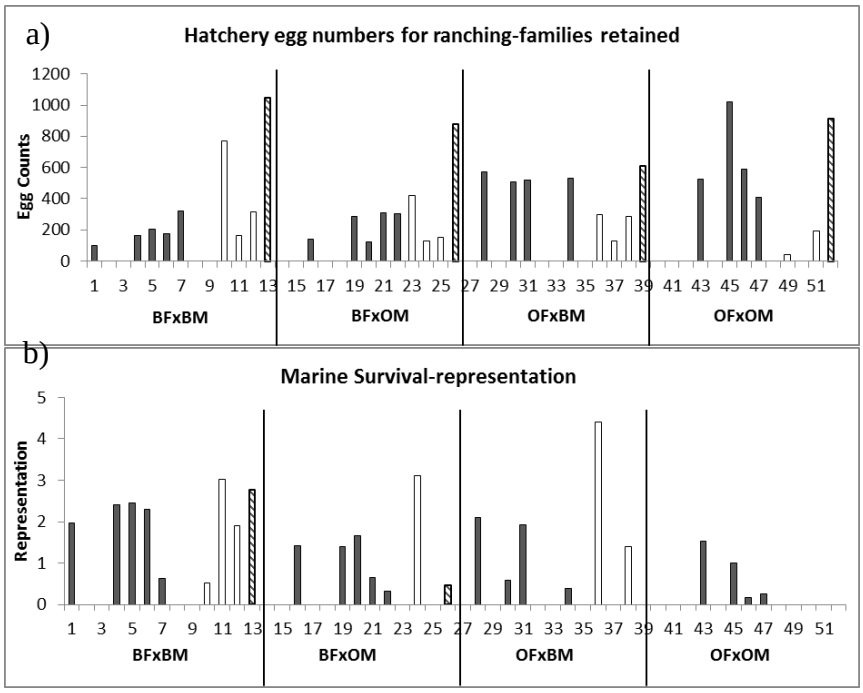

Figure 8: a) Eyed eggs retained in the hatchery for ranching, shown by group. b) Surviving marine returns corrected with egg numbers, shown by stripping date and group. Stripping one (grey bars, 34 families) were outplanted on the 22nd February 2009, second stripping (white bars, 14 families) outplanted on the 29th February, and the third stripping (striped bar March 2009, four families). BF=Burrishoole Female, BM=Burrishoole Male, OF=Owenmore Female, OM=Owenmore Male.

Adult Atlantic salmon (n=144) returned from the ocean after one and two winters at sea (1SW and 2SW) with 93% being 1SW. The overall combined survival from smolt to returning adult in 2011 and 2012 was 1.6%. This compares with the survival for Burrishoole wild smolt to adult 1SW, which was 7.5% in 2011 and probably more relevantly with Burrishoole hatchery reared smolts, which in 2011 was 2.7%. The relative survival values for the four groups in the experiment are provided in Table 13. Parentage assignment did not indicate any obvious link between the age of the broodstock used and the occurrence of 2SW offspring. Of this sample of 144 adult fish which returned, 138 were successfully used for genetic analysis.

There were significantly less marine returns in the OFxOM group when compared to the native BFxBM group (G-test, $p<0.001$), with marine survival of the OFxOM group only 18% (14 fish) of the survival of the native BFxBM group (60 fish, Figure 5, Table 13). This was also found to be true for the BFxOM hybrid group (G-test, $p<0.001$).which had 23% the survival of the Burrishoole wild group (18 fish). The

second hybrid group, OFxBM, had a survival rate of 78% relative to Burrishoole natives, although this difference was not statistically significant (41 fish, G-test, p=0.066, Figure 5, Table 13).

Table 13: Survival of each group at various stages and overall lifetime success. Full details given in Appendix VIII.

Lifetime success (egg to egg survival)	**Total Coll'd**	**Total 5 grps**	**Total 4 grps**	**BF X BM**	**BF X OM**	**OF X BM**	**OF X OM**	**Wild**
Freshwater								
Eyed eggs		69,363	53,823	13,740	13,362	13,417	13,304	15,540
Survival to 0+ (% survival)	3,695 (5.33)			575 (4.18)	822 (6.15)	804 (5.99)	682 (5.13)	841 (5.41)
Survival to smolt from July 2009 (% survival)		2.73		4.06	2.13	1.98	4.64	1.96
Overall freshwater survival (% survival)		**0.09**		**0.10**	**0.08**	**0.07**	**0.12**	**0.08**
Marine								
Ranched smolts (est. on eyed eggs in hatchery)			9,115	2,289	1,821	2,416	2,587	-
Ranched smolts based on 400		400	381	88	89	83	121	-
Ranched smolt release based on 400		400	9,115	2,105	2,129	1,985	2,894	-
Adults 1SW & 2SW (six samples could not be analysed)	144		138	61	19	44	14	-
(1.% survival on egg data)	1.58%		(1.51)	(2.66)	(1.04)	(1.82)	(0.54)	
(2. % survival on smolts)			(1.51)	(2.90)	(0.89)	(2.22)	(0.48)	-
Number of eggs returning based on total weight (kg) of female 1SW & 2SW			214,485	100,650	65,355	22,080	23,100	-
<u>Full Model values (eyed egg to green egg survival)</u>								
Number of eggs returning (Eggs per hat smolt x no. of wild smolts produced)			***1177***	***616 (1)***	***395 (0.64)***	***82 (0.13)***	***143 (0.23)***	-
No of eggs returning to river if migrating fish had produced smolts (hypothetical values)			***1906***	***1627 (1)***	***1041 (0.64)***	***228 (0.14)***	***464 (0.29)***	-

Length, weight and condition factor were examined for differences across the four different experimental groups for each age class (Table 14). Significant differences were found when adult return data were broken down by age, with 2SW fish being significantly longer and heavier than 1SW fish (ANOVA, $p<0.001$). Male fish were not found to be any different to female fish in terms of length, weight, condition factor or time of return (ANOVA, p=0.296, p=0.071, p=0.67 and p=0.892 respectively).

Table 14: Means and standard error of means for length (cm), weight (kg), condition factor and return date (Julian day) for 1SW and 2SW salmon, divided by sex and by group.

		Length (cm)		Weight (cm)		K factor		Julian day	
	N	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
All 1SW	124	59.60	0.38	2.10	0.05	0.98	0.01	243.65	2.55
Male	71	60.20	0.54	2.15	0.07	0.96	0.01	243.35	3.71
Female	53	58.80	0.51	2.05	0.06	1.00	0.01	244.06	3.32
All 2SW	9	71.74	1.40	3.88	0.26	1.03	0.03	183.56	5.16
Male	1	74.50	n/a	3.80	n/a	0.92	n/a	165.00	n/a
Female	8	71.40	1.54	3.89	0.29	1.06	0.03	185.88	5.23
1SW	124	59.60	0.38	2.10	0.05	0.98	0.01	243.65	2.55
BF X BM	55	58.92	0.55	2.05	0.06	0.99	0.01	233.87	3.85
BF X OM	18	60.87	1.29	2.28	0.13	0.99	0.02	256.61	7.23
OF X BM	37	59.83	0.65	2.06	0.08	0.95	0.02	246.05	3.73
OF X OM	14	60.04	1.08	2.22	0.16	1.00	0.03	259.07	6.79
2SW	9	71.74	1.40	3.88	0.26	1.03	0.03	183.56	5.16
BF X BM	5	71.18	1.88	3.92	0.36	1.08	0.04	185.20	8.60
BF X OM	0	-	-	-	-	-	-	-	-
OF X BM	4	72.45	2.35	3.83	0.42	1.00	0.03	181.50	5.81
OF X OM	0	-	-	-	-	-	-	-	-

The average weight and length of the adult 1SW returns to the traps was 2.10Kg and 59.0cm. An examination of the length and weight of returning 1SW adults captured (Table 14) shows that there were no significant differences in their size overall (Table 15). Similarly, there were no significant differences observed among the 2SW fish (Table 15). The mean weight of 2SW fish was 3.84Kg and length of 72.9cm.

Table 15: Significance levels for length (cm), weight (kg), condition factor and return date (Julian day) for 1SW (ANOVA) and 2SW (t-test as only two groups represented) salmon, divided by group.

Sample	N	Length (cm)	Weight (cm)	K factor
1SW	124	p=0.297	p=0.355	p=0.105
2SW	9	df=6, p=0.688	df=6, p=0.869	df=6, p=0.155

The overall sex ratio for 1SW fish was 0.57 to 0.43 male to female and 0.11 to 0.89 in favour of females in 2SW fish. The relative sex ratios of returning 1SW fish per group was fairly similar ranging for males from 50% (OFxOM) to 61% (BFxOM).

A comparison of 1SW fish returning to the Burrishoole traps between June and November 2011 showed a significant difference in time of return among the four

groups (G-test: p>0.002) with native Burrishoole fish returning some 33 days on average earlier than their Owenmore non-native counterparts with the two hybrid groups, with the BFxOM and OFxBM offspring, returning 23 days and 13 days later than the Burrishoole fish.

Time of return of the catchment was found to vary significantly across the four groups for the 1SW sample (ANOVA, p=0.002). Comparison (Tukey's method) found the BFxBM group returned earliest, with the OFxBM migrating back to freshwater next, followed by the BFxOM and lastly the OFxOM, with these last two groups significantly different to the native BFxBM group.

The recapture rate for the BFxBM group was 2.66% and 0.54% for the OFxOM group, a ratio of 5:1. The hybrid group marine survivals for the ranched smolts released in April 2010 were intermediate at 1.04% and 1.82% for the BFxOM and OFxBM groups respectively.

2.3.5 Overall Lifetime Success

The product of survival in freshwater and marine life-history stages can be used as a quantitative measure of overall lifetime success (Table 13). Lifetime success of introduced OFxOM fish relative to native BFxBM fish was found to be 24% of native fish. The BFxOM hybrid group had a lifetime success rate of 17% relative to Burrishoole fish and the second hybrid group OFxBM had a lifetime success of 48%. The relative survival overall and among groups (i.e. representation at the different life stages in the experimental river) is also presented in the survival history model, Table 13. Here lifetime success from, egg to egg is calculated by combining survivals from green egg to adult returns and converting 1SW and 2SW adult returns into eggs based on available fecundity weight models.

Suitable habitat for juvenile salmonids is present in the river downstream of the experiment trap and in freshwater Lough Feeagh. Thus, emigrant parr would potentially be able to survive and produce smolts. The second measure of smolt output assumed that emigrant parr had the same survival downstream as parr of the

equivalent group remaining in the experiment river, and combines the estimated number of smolts produced from these with the actual experiment-trap migrants, to give an estimate of sea-entry smolts. Under the experimental conditions where parr migrants survived downstream of the trap, the non-native OFxOM fish had a lifetime success of 35% relative to the pure native cross, the offspring of the BFxOM and OFxBM had a reduced lifetime success relative to the Burrishoole (23% and 49% respectively) of the Burrishoole pure group. This gives a combined hybrid value of 36%. Under the situation where only the smolts at the experimental trap are considered, the Owenmore had a lifetime success of 25% relative to the local population, the hybrid success was 24% and 45% respectively for BFxOM and OFxBM groups with a combined hybrid value of 34.5% of the pure Burrishoole group.

2.4 Discussion

This study found that the overall lifetime success of Atlantic salmon with Owenmore parents was 24% of those with Burrishoole parents. The result is lower than the 38% value observed for a previous experiment in the same river and reported by McGinnity *et al.* in 2004. The present study included reciprocal hybrids between the native and non-native groups, a novel aspect, allowing testing for an additive genetic basis of among population fitness differences (Gilk *et al.*, 2004, Fraser *et al.*, 2008). These reciprocal inter-population hybrids had an estimated combined average survival of 32.5% relative to the offspring of native fish (BFxOM and OFxBM). The intermediate performance of hybrids relative to better performing natives and inferior non-natives is considered indicative of genetically-based LA (Hatfield & Schluter 1999; Kawecki & Ebert 2004). For this experiment, environmental contributions to fitness differences were eliminated as far as possible by the common garden design, while the potential effects of shared early-rearing conditions were minimised by introducing families to the experiment-river as early in the life history as was practical (i.e. at the eyed-egg stage).

Freshwater life stage

The survival trajectories of fish for this study, as demonstrated by measurements taken at the various life history stages, were different to the previous study carried out by McGinnity (2004), especially in freshwater. For example, the freshwater survival to smolt stage was found to be an order of magnitude lower than what would be expected from this stream, as demonstrated in the work of McGinnity *et al.* (2004) and previous experiments on salmonids at this site (McGinnity *et al.*, 2003, de Eyto *et al.*, 2007, 2011). The effects of the temporal variations in the environment between years, and therefore the variable selection regimes experienced by the fish are likely to be important (Jonsson and Jonsson, 2009). The extreme weather event that occurred in July 2009, which severely affected the physical and biological nature of the river, is a case in point. Food (in terms of in-stream macroinvertebrates), was shown to be in substantially poorer supply in the months following the flood event and was significantly less prevalent and less diverse in the following two summers (de Eyto, 2011). It is expected, under some projected future

climate scenarios for the Burrishoole catchment, that the frequency of these types of extreme weather events will increase (Fealy *et al.*, 2012). Work on marble trout (*Salmo marmoratus)* in Slovenia, showed evidence of high mortality rates from this kind of weather event, leading to reduced genetic diversity (Pujolar *et al.*, 2011), suggesting that extreme weather effects could have important genetic consequences for populations. An estimated 35% of the juvenile salmon population were displaced from the experimental stream by the July 2009 flood. Post-flood electrofishing gave density estimates in line with those expected generally for 0+ salmon in that area of river (McGinnity *et al.*, 1997, 2004). Interestingly, the displacement of the juvenile salmon did not occur simultaneously with the flood, but actually followed some 36 hours after the event and continued for three days thereafter. This would suggest that the dislocation of young fish was not just a physical phenomenon but also had a behavioural component. It is possible that Owenmore and Burrishoole salmon, as they probably experience similar climatic conditions due to their catchments' close proximity, have similar behavioural adaptations for dealing with times of high flow (Jonsson and Jonsson, 2009). However, examination of the length and weight data for the fish migrating through the trap post flood and those remaining *in situ*, showed that those fish remaining in the stream were both significantly longer and heavier than the fish that migrated out of the flood disturbed system.

Fry dispersal behaviour was found to differ among the groups, with many more OF x OM fry captured moving downstream through the experiment-trap both prior to, and several days after, the flood event. The genetic basis for the behaviour is suggested by the intermediate expression of the trait in the hybrids. Parr migration in salmonids has been shown to be under genetic control (Raleigh, 1971). McGinnity *et al.*, (2004) also found that Owenmore parr were more likely to move downstream and speculated that this may be adaptive in the Owenmore River where the best nursery habitat is downstream of the spawning habitat, unlike the situation in the Burrishoole catchment. Unfortunately, it was not possible to monitor the subsequent survival of parr emigrants in this study to test the adaptive basis of these behavioural differences. Smolt output showed no differences across groups in this study, indicating no differential survival in the freshwater stage.

While considerable efforts were made to prevent natural spawning in the experimental stream in 2008, at least five female fish, and an undetermined number of adult males, in addition to mature male parr in the river, escaped our attention. These fish spawned in the river and consequently contributed to the juvenile population. Counts of unassigned/naturally spawned fish were present in all portions of the freshwater part of the experiment, sometimes in quite high numbers. The wild fish which were unintentionally present in the experiment were found to be highly represented among the parr migrating into the trap post-flood. This behaviour is quite different to the observed behaviour for native salmon which were artificially introduced into the river as part of the experiment. One possible explanation for this might be that the natural spawning occurred in this section of river's best gravel spawning habitat, which is located directly above the trapping facility and that the over-representation of wild spawned fish was a function of their proximity to the downstream trap.

Marine life stage

Almost five times the numbers of native fish (BF x BM) survived the marine phase compared to the non-natives (OF x OM), with the average combined hybrid survival being 50% of the native group. Therefore there was no evidence for heterosis (hybrid vigour) when the two types of hybrids were combined. Looking at the hybrids as two separate groups does show that the number of returning adults in the OF x BM group was not significantly different from the BF x BM group, and the numbers of BF x OM adult returns were not significantly different from the OF x OM group. This would seem to suggest a paternal effect on marine returns. One possible explanation is that marine survival was actually similar for the two hybrid groups but homing ability differed and had a strong paternal genetic basis, which had been previously suggested by a resciprocal study carried out by Bams (1976) on pink salmon (*Oncorhynchus gorbusha*) native and foreign populations, but not inter population hybrids, in each of two rivers. An experiment involving geographically distant pink salmon populations, and utilising inter population hybrids, found similar homing rates in hybrids as in controls (Gilk *et al.* 2004). For practical reasons, because it is hard to generate enough smolts from the wild for robust statistical analysis, the marine phase of the experiment used hatchery raised fish, which adds in the

possibility of hatchery effects on those fish that survived to smolt stage (Kawecki and Ebert, 2004, Garcia de Leaniz *et al.*, 2007).

It is only possible to speculate on the phenotypic traits that could drive the differences in marine survival seen between groups, but the initial behaviour of smolts and post-smolts during their migration to the marine environment is one possible area. Burrishoole smolts pass through a more complex system during their seaward migration, having to navigate Loughs Feeagh and Furnace before entering Clew Bay, whereas for Owenmore smolts there is a more limited estuarine passage, leading directly to sea. In addition, when Burrishoole smolts reach the sea, they move due west to the open ocean whereas from the Owenmore river, movement is first in a south-westerly direction. It has been suggested (Hansen *et al.*, 2003) that Atlantic salmon have genetically based navigation systems that ensure appropriate movement and since the highest marine mortality takes place in the immediate post-smolt period (Hansen *et al.,* 2003), any delay in moving offshore may be accompanied by increased mortality. The large marine survival differences seen between Burrishoole and Owenmore smolts released from the Burrishoole system are remarkable given that the mouths of each river system are only ~50km apart and fish from each population presumably experience very similar conditions once they move offshore.

This study also found weak evidence for genetically-based population differences in timing of adult returns, with natives returning earlier than non-natives and hybrids intermediate. Run timing has been shown previously to have a genetic component in Atlantic salmon (Hansen & Jonsson, 1991, Stewart et al., 2002, O'Malley *et al..*, 2010). Run-timing differences between different Atlantic salmon populations have also been demonstrated, both within and between catchments (Youngson, 1994, Vaha *et al.*, 2008). Recent work concluded that time of river entry for this species is likely due to a stable genetic polymorphism (Gurney *et al*., 2012). These ideas are compatible with the idea of F_1 outbreeding depression, that is, the extrinsic incompatibility between hybrids and the environments (river and sea) they experience.

These findings add to a number of studies showing marine performance differences between salmon populations (McGinnity *et al.* 1997, 2003, 2004; Gilk *et al.* 2004) and highlight the need to better understand the extent and scale of LA during the marine phase and potential linkages between freshwater and marine adaptations (Fraser *et al.* 2011). The idea that the river is where most selection occurs, which has been expressed in previous reviews (Taylor, 1991, Garcia de Leaniz *et al.*, 2007), but not observed in this study, could partly be due to the fact that selective pressures are more easily observed in the river than at sea, e.g. the impact of stream-based predators and parameters such as river water temperature and flow. Also, the intensity of selection (in terms of a general demographic effect rather than the rate of differential selection among individuals and families), tends to be higher in freshwater than in the marine environment (Quinn, 2005, Garcia de Leaniz *et al.*, 2007). For example, survival from egg to smolt is about 1%, compared to smolt to adult survival rates of approximately 10% (SRAI data). However, as Fraser *et al.* (2011) argue, the range of different pressures the fish experience in the marine environment, such as temperature variation, predation and salinity gradients and length of migrations are likely to exert the greater selection pressure on populations. The results of this study and previous work by McGinnity *et al.* (2004) appear to validate the argument of Fraser *et al.* (2011). Results such as those shown here highlight the importance of studying the complete life history of an anadromous species when looking for evidence of LA, as it is likely that specific and different adaptations are needed for both environments (Fraser and Bernatchez, 2005). If this experiment had examined only the freshwater portion of the Atlantic salmon's life-history, it would have considerably underestimated the difference in relative performance of natives and yintroduced individuals.

Interestingly, neither the traits of male parr maturity nor sea age of maturation conformed to an additive genetic model. In these cases, there was a suggestion of both paternal and maternal genetic influences in the determination of phenotypes associated with reproduction, i.e. the propensity for male parr maturation and age of maturation in sea returning adult salmon. There would appear to be a greater propensity for precocious maturation in male parr with a Burrishoole mother regardless of whether the father originated from a native or non-native father. We also found a higher incidence of a multi-sea-winter life history pattern in fish with a

Burrishoole father regardless of whether its mother was from the Burrishoole or Owenmore rivers. A high incidence of mature male parr has previously been associated with the harshness of the river environment experienced and the levels of marine survival of adult fish (Myers *et al.* 1986). A higher proportion of two sea winter and older fish (with their associated larger bodies), are known to dig deeper redds and produce larger eggs, and have also been associated with low energy, cold upland river habitats (Gurney *et al.*, 2012).

LA in salmonid fishes has been suggested as very important for the overall resilience and productivity of a variety of species. Work carried out on sockeye salmon (*Oncorhynchus nerka*) populations in the Bristol Bay area of Alaska have shown the importance of populations adapted to their local habitat (Hilborn *et al.*, 2003, Schindler *et al.*, 2010). It has been demonstrated that the continued productivity of the region overall is due to varying contributions from different areas and rivers over time, with local populations exhibiting varying life history strategies relative to their local habitat, due to environmental factors. The work presented here would agree with these studies, showing evidence of LA occurring across small spatial scales and also presenting evidence of the impact of extreme weather events on salmonid populations.

Conclusions and Management Implications

Under the assumption that LA does not operate across small spatial scales, it could be been argued that supplemental stocking into a river to increase productivity will not have a genetically negative impact if broodstock is obtained from the geographically nearest river with surplus Atlantic salmon. The results of this study disagree with this however, as it was found that lifetime fitness was much lower for non-native salmon from a catchment whose mouth is only 50km away from that of native fish. The reduced performance of hybrids relative to the native group (see also Gilk *et al.* 2004) suggests that supplemental stocking could result in cumulative reductions in mean fitness in stocked populations if non-natives successful interbreed with locally-adapted natives. The precautionary principle would then suggest that prudence and a full consideration of the risks of extrinsic outbreeding depression before proceeding with stocking, even if utilising broodstock obtained from neighbouring catchments or tributaries within the same catchment.

Our study shows the importance of examining all the various life history stages to observe differences. Partial studies, for example, just looking at the freshwater portion of the life history would not have been sufficient. It is recommended that future work has increased emphasis on researching LA of Atlantic salmon in the marine or coastal environment.

In terms of future work, it would be desirable to run the experiment into F_2, F_3 and further generations to ascertain the possible effect of outbreeding depression on fitness. Previous work by McGinnity *et al.* (2003), investigating the relative fitness of the offspring of farm salmon and their various hybrids compared to wild Burrishoole salmon, showed clear evidence of outbreeding depression in F_2 hybrids in the early developmental stage when examining survival rates between native and farmed fish. Outbreeding depression can be more evident in the F_2 generation for locally adapted populations (Dobzhansky, 1950, Lynch and Walsh, 1998, Beebee and Rowe, 2004). Also, if it had been possible to undertake a full reciprocal transfer experiment including the Owenmore River at the same time as running the study in the Burrishoole catchment, this would have been more ideal (Fraser *et al.*, 2011).

This study shows the importance of maintaining distinct genetic composition of populations of Atlantic salmon, even those in neighbouring catchments and apparently similar habitats. If the differences in survival found here are typical for Atlantic salmon, then supplemental stocking is likely to have a negative long term effect on productivity.

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Appendix I, Chapter 2: Broodstock details

Burrishoole Females

Sample Code	**BF_1**	**BF_2**	**BF_3**	**BF_4**	**BF_5**	**BF_6**	**BF_7**	**BF_8**	**BF_9**	**BF_11**	**BF_12**	**BF_13**	**BF_14**
Date Stripped					22/12/2008					29/12/2008		14/01/2009	
Floy Tag	Y1035	Y1033	Y1034	Y1028	Y1031	Y1032	Y1027	Y1030	Y1029	Y2122	Y2120	Y2275	Y2121
Length (cm)	60	77.2	59.2	73.2	57.5	63.1	60.4	63.5	57.7	64.5	81	74	56.3
Age	1SW	2SW	1SW	2SW	1SW	1SW	1SW	2SW	1SW	2SW	2SW	2SW	1SW
n(eggs) crossed with Burrishoole male	1547	1807	1400	1533	508	1711	1325	722	1160	1800	2336	3381	1867
n(eggs) crossed with Owenmore male	1516	1803	1400	1500	462	1733	1244	739	1173	1600	2136	3333	1667
Total n(eggs) stripped	3063	3610	2800	3033	970	3444	2569	1461	2333	3400	4472	6714	3534
n(eggs) retained in cavity*	289	234	323	346	77	582	127	277	141	259	581	217	68
TOTAL n(eggs) per hen	**3352**	**3844**	**3123**	**3379**	**1047**	**4026**	**2696**	**1738**	**2474**	**3659**	**5053**	**6931**	**3602**
Volume of 200eggs (mls)	47.5	61	45	56.5	39	45	46.5	46	37.5	45	44	42	42

Burrishoole Males

Sample Code	**BM_2**	**BM_3**	**BM_4**	**BM_5**	**BM_7**	**BM_8**	**BM_10**	**BM_11**	**BM_12**	**BM_13**	**BM_14**
Stripping Date			22/12/2008				14/01/2009		29/12/2008		
Floy Tag	Y1013	Y1016	Y1022	Y1024	Y1025	Y1012	Y2276	Y1020	Y1015	Y1014	Y1019
Length (cm)	60.4	61	60.8	63.4	62.5	55.8	62.5	60.3	54.8	52.8	61.5
Age	1SW	1SW	1SW	1SW	1SW	1SW	1SW	1SW	1SW	1SW	1SW

Owenmore Females													
Sample Code	**OF_1**	**OF_2**	**OF_3**	**OF_4**	**OF_5**	**OF_6**	**OF_7**	**OF_8**	**OF_9**	**OF_10**	**OF_11**	**OF_12**	**OF_13**
Stripping Date				**22/12/2008**						**29/12/2008**			**14/01/2009**
Floy Tag	R2250	R2249	R2248	R2247	R2246	R2245	R2244	R2243	R2234	R2233	R2232	R2231	R2997
Length (cm)	70.2	64.5	62.4	70.5	68	64.5	77.8	60.4	61	64.9	62	60	64.5
Age	2SW	1SW	1SW	2SW	2SW	1SW	2SW	1SW	1SW	1SW	1SW	1SW	1SW
n(eggs) crossed with Owenmore male	1904	2333	2064	1756	1618	1863	3719	1791	1615	2291	1521	1884	2089
n(eggs) crossed with Burrishoole male	2000	2044	2091	1963	1636	1708	3667	1909	1463	2136	1358	1737	2044
Total n(eggs) stripped	3904	4377	4155	3719	3254	3571	7386	3700	3078	4427	2879	3621	4133
n(eggs) retained in cavity	192	257	115	415	189	258	264	205	201	217	168	175	40
Total n(eggs) per hen	4096	4634	4270	4134	3443	3829	7650	3905	3279	4644	3047	3796	4173
volume of 200eggs (mls)	54	45	44	54	55	44.5	54	44	41	44	53	38	45

Owenmore Males													
Sample Code	**OM_1**	**OM_2**	**OM_3**	**OM_4**	**OM_5**	**OM_6**	**OM_7**	**OM_8**	**OM_10**	**OM_11**	**OM_12**	**OM_13**	**OM_14**
Stripping Date				22/12/2008						29/12/2008			14/01/2009
Floy Tag	R2243	R2242	R2241	R2240	R2239	R2238	R2237	R2236	R2230	R2229	R2228	R2227	R2996
Length (cm)	64.6	68.5	64	73.1	58	68.2	66.8	61	74	84.5	65.1	62.2	76
Age	1SW	1SW	1SW	2SW	1SW	1SW	1SW	1SW	2SW	2SW	1SW	1SW	2SW

Appendix II, Chapter 2: Stripping details

Table 1: Total eggs stripped, total eyed eggs, mortalities between the two stages and % survival from green to eyed eggs are given below in terms of families (showing parents used in the creation of each family) and groups, divided by stripping dates.

Group	Family	Female Parent	Male Parent	Stripping Date	Total eggs stripped	Mortalities green to eyed egg	Percentage Survival per family	Percentage survival per stripping	Total eyed eggs
BF x BM	1	**BF_4**	**BM_2**	22-Dec-08	1533	377	75.41		1156
	2	**BF_5**	**BM_3**	22-Dec-08	508	25	95.08		483
	3	**BF_2**	**BM_4**	22-Dec-08	1807	685	62.09		1122
	4	**BF_1**	**BM_5**	22-Dec-08	1547	38	97.54		1509
	5	**BF_3**	**BM_7**	22-Dec-08	1400	158	88.71	84.4	1242
	6	**BF_7**	**BM_8**	22-Dec-08	1325	587	55.7		738
	7	**BF_6**	**BM_11**	22-Dec-08	1711	65	96.2		1646
	8	**BF_8**	**BM_12**	22-Dec-08	722	45	93.77		677
	9	**BF_9**	**BM_13**	22-Dec-08	1160	57	95.09		1103
	10	**BF_12**	**BM_2**	29-Dec-08	2336	125	94.65		2211
	11	**BF_14**	**BM_14**	29-Dec-08	1867	57	96.95	94.68	1810
	12	**BF_11**	**BM_3**	29-Dec-08	1800	136	92.44		1664
	13	**BF_13**	**BM_10**	14-Jan-09	3381	313	90.74	90.76	3068
	Average						**87.27 (SE=±3.85)**		
	Total				**21097**	**2668**			**18429**
BF x OM	14	**BF_5**	**OM_1**	22-Dec-08	462	43	90.69		419
	15	**BF_9**	**OM_2**	22-Dec-08	1173	59	94.97		1114
	16	**BF_3**	**OM_3**	22-Dec-08	1400	598	57.29		802
	17	**BF_8**	**OM_4**	22-Dec-08	739	53	92.83		686
	18	**BF_7**	**OM_5**	22-Dec-08	1244	355	71.46	84.63	889
	19	**BF_1**	**OM_6**	22-Dec-08	1516	145	90.44		1371
	20	**BF_4**	**OM_7**	22-Dec-08	1500	306	79.6		1194
	21	**BF_6**	**OM_8**	22-Dec-08	1733	95	94.52		1638
	22	**BF_2**	**OM_9**	22-Dec-08	1803	182	89.91		1621
	23	**BF_11**	**OM_10**	29-Dec-08	2136	49	97.91		2087
	24	**BF_12**	**OM_11**	29-Dec-08	1600	122	92.38	95.95	1478
	25	**BF_14**	**OM_13**	29-Dec-08	1667	37	97.78		1630
	26	**BF_13**	**OM_14**	14-Jan-09	3333	218	93.46	93.46	3115
	Average						**87.92 (SE=±3.26)**		
	Total				**20306**	**2262**			**18044**

Table 1 continued:

Group	Family	Female Parent	Male Parent	Stripping Date	Total eggs stripped	Mortalities green to eyed egg	Percentage Survival per family	Percentage survival per stripping	Total eyed eggs
OF x BM	27	**OF_8**	**BM_2**	22-Dec-08	1909	355	81.4.	84.65	1554
	28	**OF_3**	**BM_3**	22-Dec-08	2091	269	87.14		1822
	29	**OF_2**	**BM_4**	22-Dec-08	2044	378	81.51		1666
	30	**OF_7**	**BM_7**	22-Dec-08	3667	148	95.96		3519
	31	**OF_4**	**BM_8**	22-Dec-08	1963	343	82.53		1620
	32	**OF_6**	**BM_11**	22-Dec-08	1708	466	72.72		1242
	33	**OF_5**	**BM_12**	22-Dec-08	1636	249	84.78		1387
	34	**OF_1**	**BM_13**	22-Dec-08	2000	176	91.2		1824
	35	**OF_9**	**BM_2**	29-Dec-08	1463	711	51.4	71.9	752
	36	**OF_10**	**BM_14**	29-Dec-08	2136	316	85.21		1820
	37	**OF_11**	**BM_3**	29-Dec-08	1358	317	76.66		1041
	38	**OF_12**	**BM_5**	29-Dec-08	1737	446	74.32		1291
	39	**OF_13**	**BM_10**	14-Jan-09	2044	122	94.03	94.03	1922
	Average						**81.45 (SE=±3.18)**		
	Totals				**25756**	**4296**			**21460**
OF x OM	40	**OF_6**	**OM_1**	22-Dec-08	1708	466	72.72	80.06	1242
	41	**OF_5**	**OM_2**	22-Dec-08	1618	181	88.81		1437
	42	**OF_4**	**OM_3**	22-Dec-08	1756	557	68.28		1199
	43	**OF_3**	**OM_4**	22-Dec-08	2064	167	91.91		1897
	44	**OF_2**	**OM_5**	22-Dec-08	2333	792	66.05		1541
	45	**OF_7**	**OM_6**	22-Dec-08	1904	221	88.39		1683
	46	**OF_1**	**OM_7**	22-Dec-08	1863	486	73.91		1377
	47	**OF_8**	**OM_8**	22-Dec-08	1791	172	90.4		1619
	48	**OF_11**	**OM_10**	29-Dec-08	1615	304	81.18	70.13	1311
	49	**OF_9**	**OM_11**	29-Dec-08	1521	485	68.11		1036
	50	**OF_12**	**OM_12**	29-Dec-08	1884	708	62.42		1176
	51	**OF_10**	**OM_13**	29-Dec-08	2291	715	68.79		1576
	52	**OF_13**	**OM_14**	14-Jan-09	2089	207	90.09	90.09	1882
	Average						**77.77 (SE=±3.03)**		
	Totals				**24437**	**5461**			**18976**

Table 2: Number of eggs outplanted in the experimental stream, retained in the hatchery for on growing as smolts, and retained for surplus group (later discarded) for each family at each stripping date.

Group	Family	Female Parent	Male Parent	Date planted in river	Number eggs planted	Number eggs retained for ranching	Number eggs retained for surplus group
BF x BM	1	**BF_4**	**BM_2**	11-Feb-09	1054	102	43
	2	**BF_5**	**BM_3**	11-Feb-09	451	0	146
	3	**BF_2**	**BM_4**	11-Feb-09	1066	0	93
	4	**BF_1**	**BM_5**	11-Feb-09	1276	166	0
	5	**BF_3**	**BM_7**	11-Feb-09	1022	204	0
	6	**BF_7**	**BM_8**	11-Feb-09	609	174	0
	7	**BF_6**	**BM_11**	11-Feb-09	1302	321	88
	8	**BF_8**	**BM_12**	11-Feb-09	631	0	156
	9	**BF_9**	**BM_13**	11-Feb-09	1089	0	146
	10	**BF_12**	**BM_2**	20-Feb-09	1272	772	0
	11	**BF_14**	**BM_14**	20-Feb-09	1250	166	0
	12	**BF_11**	**BM_3**	20-Feb-09	1333	314	0
	13	**BF_13**	**BM_10**	01-Mar-09	1285	1047	928
	Total					**13640**	**3266**
BF x OM	14	**BF_5**	**OM_1**	11-Feb-09	380	0	0
	15	**BF_9**	**OM_2**	11-Feb-09	1027	0	129
	16	**BF_3**	**OM_3**	11-Feb-09	720	0	142
	17	**BF_8**	**OM_4**	11-Feb-09	609	0	82
	18	**BF_7**	**OM_5**	11-Feb-09	826	0	58
	19	**BF_1**	**OM_6**	11-Feb-09	1020	285	0
	20	**BF_4**	**OM_7**	11-Feb-09	1018	120	0
	21	**BF_6**	**OM_8**	11-Feb-09	1318	309	100
	22	**BF_2**	**OM_9**	11-Feb-09	1300	306	53
	23	**BF_11**	**OM_10**	20-Feb-09	1255	418	744
	24	**BF_12**	**OM_11**	20-Feb-09	1304	129	0
	25	**BF_14**	**OM_13**	20-Feb-09	1250	152	0
	26	**BF_13**	**OM_14**	01-Mar-09	1285	880	1095
	Total				**13312**	**2599**	**2403**

Table 2 continued:

Group	Family	Female Parent	Male Parent	Date planted in river	Number eggs planted	Number eggs retained for ranching	Number eggs retained for surplus group
OF x BM	27	**OF_8**	**BM_2**	11-Feb-09	1000	0	666
	28	**OF_3**	**BM_3**	11-Feb-09	1000	571	419
	29	**OF_2**	**BM_4**	11-Feb-09	1023	0	828
	30	**OF_7**	**BM_7**	11-Feb-09	1020	510	2275
	31	**OF_4**	**BM_8**	11-Feb-09	1000	520	248
	32	**OF_6**	**BM_11**	11-Feb-09	1000	0	318
	33	**OF_5**	**BM_12**	11-Feb-09	1019	0	453
OF x BM	34	**OF_1**	**BM_13**	11-Feb-09	1037	528	351
	35	**OF_9**	**BM_2**	20-Feb-09	710	0	0
	36	**OF_10**	**BM_14**	20-Feb-09	1285	295	380
	37	**OF_11**	**BM_3**	20-Feb-09	926	130	0
	38	**OF_12**	**BM_5**	20-Feb-09	1000	285	0
	39	**OF_13**	**BM_10**	01-Mar-09	1260	608	0
	Totals				**13280**	**3447**	**5938**
	40	**OF_6**	**OM_1**	11-Feb-09	1000	0	372
	41	**OF_5**	**OM_2**	11-Feb-09	1035	0	428
	42	**OF_4**	**OM_3**	11-Feb-09	1020	0	330
	43	**OF_3**	**OM_4**	11-Feb-09	1000	524	352
	44	**OF_2**	**OM_5**	11-Feb-09	1023	0	698
	45	**OF_7**	**OM_6**	11-Feb-09	1020	1019	1321
	46	**OF_1**	**OM_7**	11-Feb-09	1056	592	122
OF x OM	47	**OF_8**	**OM_8**	11-Feb-09	1000	409	338
	48	**OF_11**	**OM_10**	20-Feb-09	975	0	0
	49	**OF_9**	**OM_11**	20-Feb-09	896	44	0
	50	**OF_12**	**OM_12**	20-Feb-09	974	0	0
	51	**OF_10**	**OM_13**	20-Feb-09	1255	190	0
	52	**OF_13**	**OM_14**	01-Mar-09	1000	913	0
	Average						
	Totals				**13254**	**3691**	**3961**

Appendix III, Chapter 2: Survival of subsampled eyed eggs retained in the hatchery from all families outplanted in the experimental river.

Table 1: Survival of subsampled eyed eggs retained in the hatchery from all families outplanted in the experimental river.

Vibert Box No.	Stripping Date	Female Parent	Male Parent	Starting n(fry)	n(morts) eggs	Date all hatched	n(morts) alveins	n(morts) total	n(with deformities)
1A	22/12/2008	OF_6	OM_1	25	0	18/03/2009	0	0	0
1B	22/12/2008	OF_5	OM_2	25	1	20/03/2009	0	1	0
2A	22/12/2008	OF_4	OM_3	25	0	20/03/2009	0	0	0
2B	22/12/2008	OF_3	OM_4	25	0	20/03/2009	0	0	0
3A	22/12/2008	OF_2	OM_5	25	0	20/03/2009	0	0	0
3B	22/12/2008	OF_7	OM_6	25	1	20/03/2009	1	2	0
4A	22/12/2008	OF_1	OM_7	25	1	20/03/2009	0	1	0
4B	22/12/2008	OF_8	OM_8	25	0	20/03/2009	0	0	0
5A	22/12/2008	OF_8	BM_2	25	0	20/03/2009	0	0	0
5B	22/12/2008	OF_3	BM_3	25	0	20/03/2009	0	0	0
6A	22/12/2008	OF_2	BM_4	25	0	23/03/2009	0	0	0
6B	22/12/2008	OF_7	BM_7	25	0	23/03/2009	0	0	0
7A	22/12/2008	OF_4	BM_8	25	0	23/03/2009	0	0	0
7B	22/12/2008	OF_6	BM_11	25	0	23/03/2009	0	0	0
8A	22/12/2008	OF_5	BM_12	25	0	20/03/2009	0	0	0
8B	22/12/2008	OF_1	BM_13	25	0	20/03/2009	0	0	0
9A	22/12/2008	BF_4	BM_2	25	0	18/03/2009	0	0	0
9B	22/12/2008	BF_5	BM_3	25	3	20/03/2009	0	3	0

Table 1 continued

Vibert No.	Box	Stripping Date	Female Parent	Male Parent	Starting n(fry)	n(morts) eggs	Date all hatched	n(morts) alveins	n(morts) total	n(with deformities)
10A		22/12/2008	**BF_2**	**BM_4**	25	0	20/03/2009	0	0	0
10B		22/12/2008	**BF_1**	**BM_5**	25	0	20/03/2009	0	0	0
11A		22/12/2008	**BF_3**	**BM_7**	25	5	20/03/2009	0	5	0
11B		22/12/2008	**BF_7**	**BM_8**	25	0	20/03/2009	0	0	0
12A		22/12/2008	**BF_6**	**BM_11**	25	7	20/03/2009	0	7	0
12B		22/12/2008	**BF_8**	**BM_12**	25	0	20/03/2009	0	0	0
13A		22/12/2008	**BF_9**	**BM_13**	25	0	23/03/2009	0	0	0
13B		22/12/2008	**BF_5**	**OM_1**	25	0	18/03/2009	0	0	1 curved spine
14A		22/12/2008	**BF_9**	**OM_2**	25	0	23/03/2009	0	0	1 curved spine
14B		22/12/2008	**BF_3**	**OM_3**	25	0	20/03/2009	0	0	0
15A		22/12/2008	**BF_8**	**OM_4**	25	1	20/03/2009	0	1	0
15B		22/12/2008	**BF_7**	**OM_5**	25	0	20/03/2009	0	0	0
16A		22/12/2008	**BF_1**	**OM_6**	25	0	20/03/2009	0	0	0
16B		22/12/2008	**BF_4**	**OM_7**	25	0	20/03/2009	1	1	0
17A		22/12/2008	**BF_6**	**OM_8**	25	1	18/03/2009	0	1	0
17B		22/12/2008	**BF_2**	**OM_9**	25	0	20/03/2009	0	0	0
18A		29/12/2008	**OF_11**	**OM_10**	25	0	25/03/2009	0	0	0
18B		29/12/2008	**OF_9**	**OM_11**	25	0	27/03/2009	0	0	0
19A		29/12/2008	**OF_12**	**OM_12**	25	0	25/03/2009	0	0	0
19B		29/12/2008	**OF_10**	**OM_13**	25	0	25/03/2009	0	0	0
20A		29/12/2008	**OF_9**	**BM_2**	25	0	25/03/2009	0	0	0
20B		29/12/2008	**OF_10**	**BM_14**	25	0	25/03/2009	0	0	0
21A		29/12/2008	**OF_11**	**BM_3**	25	0	25/03/2009	0	0	0
21B		29/12/2008	**OF_12**	**BM_5**	25	0	25/03/2009	0	0	0
22A		29/12/2008	**BF_12**	**BM_2**	25	0	27/03/2009	0	0	1 curved spine
22B		29/12/2008	**BF_14**	**BM_14**	25	0	27/03/2009	1	1	0
23A		29/12/2008	**BF_11**	**BM_3**	25	2	27/03/2009	1	3	0
23B		29/12/2008	**BF_11**	**OM_10**	25	5	25/03/2009	0	5	0
24A		29/12/2008	**BF_12**	**OM_11**	25	5	27/03/2009	0	5	0
24B		29/12/2008	**BF_14**	**OM_13**	25	0	27/03/2009	0	0	0
25A		14/01/2009	**BF_13**	**BM_10**	25	0	22/04/2009	0	0	0
25B		14/01/2009	**BF_13**	**OM_14**	25	0	22/04/2009	0	0	0
26A		14/01/2009	**OF_13**	**OM_14**	25	0	22/04/2009	11	11	0
26B		14/01/2009	**OF_13**	**BM_10**	25	0	22/04/2009	24	24	0

Appendix IV, Chapter 2: PCR recipes

a) PCR recipes for Licor analysis of salmon samples, all values in µl:

197, 171, 3016 multiplex:

	x1	x110
5x Green	0.5	55
5x White	1.5	165
$MgCl_2$	0.2	22
DNTPs	2	220
Ssa197 forward	0.05	5.5
Ssa197 reverse	0.05	5.5
Ssa171 forward	0.2	22
Ssa171 reverse	0.2	22
Ssp3016 forward	0.2	22
Ssp3016 reverse	0.2	22
Taq	0.1	11
H_2O	3.8	418

Mhc1, Sosl85 Multiplex:

	x1	x110
5x Green	0.5	55
5x White	1.5	165
$MgCl_2$	0.2	22
DNTPs	2	220
Mhc1 forward	0.025	2.75
Mhc1 reverse	0.025	2.75
Sosl85 forward	0.1	11
Sosl85 reverse	0.1	11
Taq	0.1	11
H_2O	4.45	489.5

Mhc2, Ssa170 multiplex:

	x1	x110
5x Green	0.5	55
5x White	1.5	165
$MgCl_2$	0.2	22
DNTPs	2	220
Mhc2 forward	0.05	5.5
Mhc2 reverse	0.05	5.5
Ssa170 forward	0.05	5.5
Ssa170 reverse	0.05	5.5
Taq	0.1	11
H_2O	4.5	495

SsaD71, Ssp2216 multiplex:

	x1	x110
5x Green	0.5	55
5x White	1.5	165
$MgCl_2$	0.2	22
DNTPs	2	220
SsaD71 forward	0.2	22
SsaD71 reverse	0.2	22
Ssp2216 forward	0.025	2.75
Ssp2216 reverse	0.025	2.75
Taq	0.1	11
H_2O	4.25	467.5

Ssp2210:

	x1	x110
5x Green	0.5	55
5x White	1.5	165
$MgCl_2$	0.2	22
DNTPs	2	220
Ssp2210 forward	0.1	11
Ssp2210 reverse	0.1	11
Taq	0.1	11
H_2O	4.5	495

b) PCR recipes for ABI analysis of salmon samples, all values in µl:

Panel 1:

	x1	x110
Master Mix	1.75	192.5
Ssa289 forward	0.056	6.16
Ssa289 reverse	0.056	6.16
Ssa197 forward	0.07	7.7
Ssa197 reverse	0.07	7.7
Mhc2 forward	0.014	1.54
Mhc2 reverse	0.014	1.54
Ssa404 forward	0.021	2.31
Ssa404 reverse	0.021	2.31
H_20	0.554	60.94

Panel 2:

	x1	x110
Master Mix	1.75	192.5
Ssa442 forward	0.021	2.31
Ssa442 reverse	0.021	2.31
Ssp2201 forward	0.021	2.31
Ssp2201 reverse	0.021	2.31
Ssa408 forward	0.028	3.08
Ssa408 reverse	0.028	3.08
One9Asc forward	0.014	1.54
One9Asc reverse	0.014	1.54
SSaD71 forward	0.028	3.08
SSaD71 reverse	0.028	3.08
Mhc1 forward	0.021	2.31
Mhc1 reverse	0.021	2.31
H_20	0.484	53.24

Panel 3:

	x1	x110
Master Mix	1.75	192.5
SsaD48 forward	0.014	1.54
SsaD48 reverse	0.014	1.54
Ssp2215 forward	0.007	0.77
Ssp2215 reverse	0.007	0.77
Ssa43 forward	0.014	1.54
Ssa43 reverse	0.014	1.54
Ssa407 forward	0.056	6.16
Ssa407 reverse	0.056	6.16
Ssp3016 forward	0.014	1.54
Ssp3016 reverse	0.014	1.54
Ssa410 forward	0.07	7.7
Ssa410 reverse	0.07	7.7
H_20	0.4	44

Panel 4:

	x1	x110
Master Mix	1.75	192.5
Ssa202 forward	0.021	2.31
Ssa202 reverse	0.021	2.31
Ssp2210 forward	0.0105	1.155
Ssp2210 reverse	0.0105	1.155
SsaD170 forward	0.021	2.31
SsaD170 reverse	0.021	2.31
Ssa171 forward	0.021	2.31
Ssa171 reverse	0.021	2.31
H_20	0.603	66.33

Panel 5:

	x1	x110
Master Mix	1.75	192.5
Ssa417 forward	0.014	1.54
Ssa417 reverse	0.014	1.54
Ssp2216 forward	0.007	0.77
Ssp2216 reverse	0.007	0.77
Sosl85 forward	0.042	4.62
Sosl85 reverse	0.042	4.62
SsaD157 forward	0.042	4.62
SsaD157 reverse	0.042	4.62
Ssp1605 forward	0.021	2.31
Ssp1605 reverse	0.021	2.31
H20	0.498	54.78

Appendix V, Chapter 2: Summary statistics for population genetics parameters for eight loci across parental samples used.

Table 1: Summary statistics for *S. salar* samples screened for 8 neutral microsatellite loci for parental groups: ***N*** = number of individuals screened per sample: ***A*** (***T%***)= number of alleles (% observed in sample in relation to total observed among all samples), $\boldsymbol{H_o}$ = observed heterozygosity, $\boldsymbol{H_e}$ = expected heterozygosity (Nei, 1987), ***A*r** = Allelic richness following the rarefaction method (Petit *et al*., 1998), **HWE** = Significance of exact tests for non-conformance to Hardy-Weinberg Expectations (Guo and Thompson, 1992), ns=not significant. **HWE-R** = *P* values of exact tests for non-conformance to Hardy-Weinberg Expectations. Significant values after Bonferroni correction (0.05/8=0.00625) are given in bold. Average values per loci are also provided.

Sample		SSp2210	SSp2216	SSa171	Ssa3016	SSa197	SSOSL85	SSa170	SSaD71	Avg.
Burrishoole Female	N	13	13	13	13	13	13	13	13	13.0
	A	4	12	8	7	9	10	14	12	9.5
	At%	40.0	80.0	57.1	50.0	47.4	62.5	56.0	38.7	52.1
	A_R	4.0	12.0	8.0	7.0	9.0	10.0	14.0	12.0	-
	H_O	0.582	0.932	0.819	0.659	0.859	0.895	0.923	0.923	0.8
	H_E	0.539	0.923	0.846	0.769	0.692	0.769	1.000	1.000	0.8
	HWE	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	
Burrishoole Male	N	13	13	13	13	13	13	13	13	13.0
	A	7	11	6	8	9	9	13	11	9.3
	At%	70.0	73.3	42.9	57.1	47.4	56.3	52.0	35.5	50.7
	A_R	7.0	11.0	6.0	8.0	9.0	9.0	13.0	11.0	-
	H_O	0.766	0.914	0.775	0.812	0.865	0.892	0.886	0.917	0.853
	H_E	0.769	0.923	0.615	0.923	0.846	0.846	0.846	1.000	0.846
	HWE	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	
Owenmore Female	N	13	13	13	13	13	13	13	13	13.0
	A	7	12	10	10	11	11	18	16	11.9
	At%	70.0	80.0	71.4	71.4	57.9	68.8	72.0	51.6	65.1
	A_R	7.0	12.0	10.0	10.0	11.0	11.0	18.0	16.0	-
	H_O	0.852	0.886	0.865	0.892	0.905	0.902	0.966	0.960	0.903
	H_E	0.846	1.000	0.769	0.769	0.923	0.769	1.000	1.000	0.885
	HWE	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	
Owenmore Male	N	13	13	13	13	13	13	13	13	13.0
	A	9	12	11	12	11	10	13	19	12.1
	At%	90.0	80.0	78.6	85.7	57.9	62.5	52.0	61.3	66.4
	A_R	9.0	12.0	11.0	12.0	11.0	10.0	13.0	19.0	-
	H_O	0.825	0.917	0.855	0.917	0.908	0.892	0.948	0.975	0.905
	H_E	0.923	0.846	0.923	0.846	0.846	0.769	0.769	0.923	0.856
	HWE	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	
Locus Average over samples	N	13	13	13	13	13	13	13	13	13.0
	A	7	12	9	9	10	10	15	15	10.7
	At%	67.5	78.3	62.5	66.1	52.6	62.5	58.0	46.8	58.6
	A_R	6.8	11.8	8.8	9.3	10.0	10.0	14.5	14.5	-
	H_O	0.756	0.912	0.828	0.820	0.884	0.895	0.931	0.944	0.871
	H_E	0.769	0.923	0.788	0.827	0.827	0.788	0.904	0.981	0.851

Appendix VI, Chapter 2: Means and SE for Length, Weight and C.F. for all groups

Table 1:Means and standard errors for length (cm), weight (g) and condition factor values for all groups and all sampling stages of the freshwater phase of the experiment.

Preflood		**Length (cm)**		**Weight (g)**		**K factor**	
	Count	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
BF X BM	28	3.274	0.098	0.357	0.044	0.964	0.038
OF X BM	44	3.789	0.613	0.309	0.013	0.933	0.032
BF X OM	45	3.116	0.054	0.300	0.015	1.012	0.052
OF X OM	83	3.112	0.032	0.306	0.009	1.020	0.022
WILD	97	3.110	0.033	0.281	0.010	0.934	0.023
Flood							
BF X BM	72	3.987	0.047	0.675	0.027	1.033	0.016
OF X BM	101	3.996	0.035	0.697	0.020	1.071	0.014
BF X OM	94	4.069	0.039	0.712	0.025	1.025	0.017
OF X OM	109	4.085	0.039	0.749	0.022	1.082	0.017
WILD	66	3.994	0.042	0.684	0.024	1.056	0.021
Electrofishing							
BF X BM	22	4.3864	0.0896	0.8745	0.0485	1.0212	0.0197
OF X BM	29	4.3207	0.0567	0.8352	0.0385	1.0161	0.0202
BF X OM	33	4.3727	0.0639	0.8473	0.0425	0.9893	0.0163
OF X OM	22	4.3273	0.0985	0.8373	0.0524	1.0144	0.0307
WILD	39	4.2974	0.0579	0.7838	0.0343	0.9658	0.0128
Precocious males							
BF X BM	6	11.767	0.448	19.43	1.84	1.1807	0.0361
OF X BM	3	12.533	0.722	25.94	5.43	1.2742	0.0528
BF X OM	7	11.686	0.363	19.58	1.11	1.2344	0.0568
OF X OM	5	11.18	0.443	17.19	1.88	1.215	0.0397
WILD	8	11.533	0.388	19.92	2.04	1.2788	0.0554
Smolts							
BF X BM	8	12.4	0.559	20.41	2.4	1.0293	0.0216
OF X BM	6	12.5	0.326	19.95	1.31	1.0168	0.0189
BF X OM	4	13.45	0.352	25.45	2.05	1.041	0.0327
OF X OM	11	12.836	0.164	21.73	1.05	1.0193	0.016
WILD	4	11.725	0.852	16.76	2.71	1.0195	0.0442

Appendix VII, Chapter 2: Electrofishing details

Locations and number of sites, area of sites, counts of three fishings and total catch for quantitative electrofishing that occurred on three dates in the experimental stretch of the Srahrevagh River during the duration of the experiment.

River	Species	Age	Site	Date sampled	x	y	Area (m^2)	1st fishing	2nd fishing	3rd fishing	Total
	July 2009 Sample										
Rough River	Salmon	0+	1	10Jul09	97690	304852	109.67	13	11	7	31
			2	10Jul09	97915	304908	98.47	14	13	4	31
			3	09Jul09	97200	304782	172.5	27	14	3	44
			4	09Jul09	97400	304783	155.4	33	15	3	51
			5	10Jul09	98005	304954	140.8	10	5	4	19
	August 2009 Sample										
Rough River	Salmon	0+	1	23Sep09	97690	304852	109.664	9	10	4	23
			2	23Sep09	97915	304908	96.6	10	6	0	16
			3	23Sep09	97200	304782	181	19	12	5	36
			4	23Sep09	97400	304783	129.5	9	10	1	20
			5	23Sep09	98005	304954	136.4	11	9	1	21
	August 2010 Sample										
Rough River	Salmon	1+	1	27Aug10	97690	304852	127.4	9	3	2	14
			2	27Aug10	97915	304908	117.8	7	5	0	12
			3	27Aug10	97200	304782	160.0	3	0	0	3
			4	27Aug10	97400	304783	98.4	5	1	3	9
			5	27Aug10	98005	304954	115.8	11	6	4	21

Appendix VIII, Chapter 2: Survival model

Table 1: Values used to estimate survival for the freshwater phase of the experiment for all groups at all stages. Percentage survival values are given in bold

Freshwater Survival	Total Collected	Total 5 groups	Total 4 groups	BF X BM	BF X OM	OF X BM	OF X OM	Wild
Eyed eggs		**69,363**	**53,823**	**13,740**	**13,362**	**13,417**	**13,304**	**15,540**
Eyed eggs (strip 1)			33,237	8,600	8,268	8,165	8,204	
Eyed eggs (strip 2)			15,756	3,855	3,809	3,992	4,100	
Eyed eggs (strip 3)			4,830	1,285	1,285	1,260	1,000	
Pre-flood migrants	412	298	200	28	45	44	83	98
Corrected numbers to 412				39	62	61	115	135
Flood migrants	1,278	442	376	72	94	111	109	66
Corrected numbers to 1,278	1,278			208	272	321	315	191
Post flood residents	176	145	106	22	33	29	22	39
Corrected numbers to 176								
Post flood residents based on July Efish	2,417	2,417		367	550	483	367	650
Flood migrants + post flood residents = in river before flood (% survival)	**3,695**	**5.33% survival to 0+**		**575 (4.18%)**	**822 (6.15%)**	**804 (5.99%)**	**682 (5.13)**	**841 (5.41%)**
Remainder in river after all samples killed	2,272			345	517	454	345	611
Ef July 2009 – Ef Sept 2009 trap migrants	541							
Post flood residents corrected Efish Sep 2009 (estimated, no genetic data)	1,573			239	358	315	239	423
Migrants Sept 2009 – Aug 2010	352							
Efish August 2010 (% surv Sept 2009 Efish to Aug 2010 Efish)	**891 (56.64%)**							
Migrants Aug 2010 to Oct 2010	43							
Combined pre-smolts & smolts	110	62	50	14	11	9	16	12
Ef 2010 to combined smolt % survival		6.96%						
Combined smolt vs. July 2009 (% surv)		**2.73%**		**4.06%**	**2.13%**	**1.98%**	**4.64%**	**1.96%**
Overall freshwater survival from eyed egg to smolt (% surv)		**0.09%**		**0.10%**	**0.08%**	**0.07%**	**0.12%**	**0.08%**

Table 2: Values used to estimate survival for the marine phase of the experiment for all groups at all stages. Percentage survival values are given in bold

Marine Survival	**Total Collected**	**Total 4 groups**	**BF X BM**	**BF X OM**	**OF X BM**	**OF X OM**
Ranched smolts (est. on eyed eggs in hatchery)		9,115	2,289	1,821	2,416	2,587
Ranched smolts based on 400	400	381	88	89	83	121
Ranched smolt release based on 400	400	9,115	2,105	2,129	1,985	2,894
Adults 1SW & 2SW (six samples could not be analysed)	**144**	**138**	61	19	44	14
1. % survival on eggs	1.58%	1.51%	2.66%	1.04%	1.82%	0.54%
2. % survival on smolts	1.58%	1.51%	2.90%	0.89%	2.22%	0.48%
Adults 1SW & 2SW(% surv on green eggs to adults)						
Adults 1SW & 2SW(% surv on eyed eggs to adults based on smolts x 100)		76	37	11	16	9
Adult 1SW returns	134	130	57	19	40	14
Number female (1SW)		54	23	6	17	7
Ave wt. female Kg 1SW		2.09	2.01	2.08	2.1	2.16
Adult 2SW (1. % surv on eggs, 2. % survival on smolts) returns	10	8	4	0	4	0
Number female (2SW)		7	4	0	3	0
Ave wt. female Kg 1SW		3.93	3.77		3.93	
Number of eggs returning based total weight (kg) of female 1SW & 2SW (hatchery smolt to egg – estimated from Burrishoole fecundity: 1,422 /Kg, Owenmore fecundity: 1632 eggs/kg)		214485	100650	65355	22080	23100
Eggs per hatchery smolt		24	44	36	9	9
Combined pre-smolts & smolts		50	14	11	9	16
Smolt output for hypothetical migrant survival		31	23	18	16	36
<u>Full Model values (eyed egg to green egg survival in total eggs)</u>						
Number of eggs returning (Shrahrevagh R. smolts– full model to give eyed egg to green egg survival) Eggs per hat smolt x no. of wild smolts		***1177***	***616***	***395***	***82***	***143***
Proportion survival by group			***1***	***0.64***	***0.13***	***0.23***
No of eggs returning to river if migrating fish had produced smolts (hypothetical values plus actual smolts through trap)		***1906***	***1627***	***1041***	***228***	***464***
Proportion survival by group(combined smolts)			***1***	***0.64***	***0.14***	***0.29***

Chapter 3:

The genetic anatomy of an invasion: a case study using *Salmo trutta* L. from Newfoundland, Canada

Salmon Cove River, Newfoundland.

Abstract

The brown trout, *Salmo trutta* L., was introduced into many areas worldwide outside its native range in the late 19th century. The species was introduced into Newfoundland, now a province of Canada, in 1883. Over the last 130 years, successful populations have been established in the environs of St. John's and have spread along the north and south coasts of the Avalon Peninsula. Naturally spawning aggregations have now established themselves in rivers up to approximately 500km from the source of the introduction. This study examined the genetic anatomy (genetic parameters), using genetic variation at 16 microsatellite DNA loci, of a successful invasion of *S. trutta* L., outside of its native range by examining contemporary samples from Scotland and Germany and samples from 12 sites across the Avalon Peninsula. The study provides evidence of the route taken by the fish (i.e. differences between the northern and southern aggregations, reduction in genetic variability at greater distance from the original introduction) and provides support for many of the historical records on the invasion event, such as establishing that the current invasive populations appear to be descended from Scottish brown trout. The Rennies River in St .John's appears to be the location of the original introduction of the species, with brown trout migrating, most likely in their anadromous form, to both the north and south of St. John's, following a classic stepping-stone model of invasion. One exception is the Salmonier River aggregation, which may show evidence of human interference in the form of unrecorded stocking. Established invasive populations show a pattern of isolation by distance and lower allelic diversity in populations located further geographically from the source of the invasion, indicating possible founder effects from small numbers of initial invaders. The results of the study are important in terms of the clear pattern visible in the genetics of the invasion. This has implications for understanding the mechanisms behind a successful invasion, and for advice on controlling and mitigating against spread of non-native species into novel areas.

3.1 Introduction

Salmonid fishes have been widely introduced throughout the globe for various purposes, mainly sport fishing and aquaculture (Valiente *et al.,* 2010). These have proved to be successful invaders in many areas (Quinn *et al.*, 2001, Ayllon *et al.,* 2004, Ayllon *et al.,* 2006, Launey *et al.*, 2010, Valiente et al., 2010). Fishes from the genera *Oncorhynchus*, *Salmo* and *Salvelinus* have all been transplanted at different times and to different areas (Young *et al.,* 2010, Westley and Fleming, 2011).

The brown trout was particularly highly prized as a sport fish by home sick settlers and over the course of approximately 90 years, spanning the 19th and 20th centuries, brown trout were intentionally introduced to a number of locations worldwide (Westley and Fleming, 2011). The main areas of introductions which have been studied for their ecological impacts, are in the southern hemisphere and include New Zealand (Quinn *et al.,* 2001, Kinnison *et al*., 2008), Chile and Argentina, the Falkland Islands, South Africa, Tasmania, Western Australia (Garcia de Leaniz *et al.*, 2010) and the Kerguelen Islands (Launey *et al.,* 2010). Brown trout and other salmonid species have been shown to outcompete native galaxiids across the southern hemisphere (Young *et al.,* 2010)

In the northern hemisphere, the first introduction of the brown trout into North America was by the US Fish Commission in 1883 in Michigan State, which was also the year that brown trout were first introduced to Newfoundland (DFO, 2010, Westley *et al.,* 2012). In the United States, brown trout has been introduced in 45 of the 50 states over the last 130 years, with a current self-sustaining population in 34 states (USGS, 2014). The brown trout is able to live and grow in warmer waters than other native trout species, such as the brook trout (*Salvelinus fontinalis,* Mitchill, 1814). As such, the brown trout has been able to establish itself in many areas in North America in which brook trout cannot exist or where warmer waters exist (USGS, 2014). The brown trout also grows faster and can potentially be bigger than native species, such as the brook trout. Therefore brown trout can outcompete brook trout and other native species (O'Connell, 1982). The food of brown trout, primarily insects, molluscs and smaller fish, is also widely available in North American rivers

and streams and does not constrain growth (O'Connell, 1982). While invasive brown trout have been extensively studied in the southern hemisphere, very little work has been done on populations in the northern hemisphere to date.

In this study, an invasive is defined as a non-native species which has been introduced to a new environment through human actions and has subsequently spread without human input. An invasive is also generally known to cause ecological damage in the new environment (Lockwood, 2005). Brown trout in Newfoundland, introduced originally by human intervention, have been spreading naturally over the last 130 years and are showing evidence of negatively impacting local populations of brook trout and possibly Atlantic salmon (Van Zyll de Jong *et al.,* 2004, Westley and Fleming, 2011).

Patterns of gene flow (in terms of migration of an invasive, in this case) can be described using two simplified models: the island model, and the stepping stone model. The island model assumes that a population is subdivided into a series of demes where migrants are equally likely to immigrate to any of the other demes. The stepping-stone model assumes that migration will be greater between demes that are near each other and can be either linear or two-dimensional (Allendorf and Luikart, 2007).

3.1.1 Recorded history of the introduction of brown trout to Newfoundland

Brown trout ova from the Howietoun hatchery, Stirling, Scotland, were initially imported to St. John's, Newfoundland, Canada in 1883 (when Newfoundland was still a colony of Great Britain), with further importations up to and including 1906 (Westley and Fleming, 2011). These ova were mainly sourced from broodstock taken from Loch Leven, Scotland.

At this time, Loch Leven brown trout were being exported to many parts of the world, including other parts of Europe, North and South America, Australia and New Zealand (Maitland, 1887, Garcia de Leaniz *et al.,* 2010). In addition, it is thought

that the German ‘von Behr’ strain, originally sourced from tributaries of the Elbe close to the city of Hamburg, was also exported to other parts of Europe, North America and Chile together with an “English” strain possibly sourced from the chalk streams in the south of England (Frost, 1940, Van Zyll de Jong *et al.*, 2004).

The first imported ova survived the trans-Atlantic crossing well (Maitland, 1887, Westley and Fleming, 2011) and were initially stocked in the land-locked Windsor Lake, which still supports a brown trout population thought to be directly descended from this original introduction (Van Zyll de Jong *et al.*, 2004). Later shipments of ova were reared in Long Pond, a lake in the environs of the city of St. John’s with sea access via Rennies River and Quidi Vidi Lake (DFO, 2010, Westley and Fleming, 2011).

Following the successful importation of brown trout ova in the years 1883, 1884, 1892 and 1905-1906, a hatchery was established at Long Pond, St. John’s and trout fry were successfully bred for a period of thirty years, from mature adults sourced from the original imported ova (Westley and Fleming 2011). The majority of brown trout introductions occurred in the St Johns area, sourced from the Loch Leven strain and it is thought that straying anadromous fish, originating from these introductions, subsequently established themselves in new catchments. A small number of introductions are also thought to have occurred outside of the St. John’s area, based on local records.

The spread of brown trout around the Avalon Peninsula beyond this was believed to be as a result of natural invasion events (Westley and Fleming, 2011), although there is a lack of strong evidence for this up to now. Data from Department of Fisheries and Oceans (DFO) surveys suggests timings for some of the more recent invasions. For example, there are no records of brown trout being present in either the South East Placentia or Port Rexton systems 20 years ago, both of which now support brown trout populations (DFO, 2010). However, before this time there are no official records kept of timings of invasion events into Newfoundland rivers and streams.

Loch Leven brown trout are a land-locked population, but they are unusual in that they display a colouration similar to that of “silvered” sea trout as adults, when they

migrate from streams to a freshwater lake. It would appear that in order to successfully spread into the large number of watersheds in Newfoundland they have resumed (or re-adapted to or re-evolved), an anadromous life history (Westley and Fleming, 2011). There are no freshwater routes that would allow for the pattern of invasion shown in brown trout on Newfoundland, and no records or other evidence of human implantation in the last 100 years, yet surveys of local rivers show continuing expansion of brown trout in Newfoundland (DFO, 2010) (It is recognised that freshwater resident *Salmo trutta* with no access to the marine environment do retain the ability to smoltify and migrate to sea (Frost and Brown, 1967)). It is thought that introduced trout started to expand into suitable catchments on the Avalon Peninsula, Newfoundland both in northerly and southerly directions from the original source of anthropogenic introductions (to a small number of waters in or close to the city of St. John's).

Westley and Fleming (2011) found that invasive brown trout in Newfoundland did not establish successfully in every available catchment, but instead were more likely to establish in larger, more productive catchments. However, their study also suggested that all catchments in Newfoundland are probably susceptible to brown trout invasion as there were no abiotic factors acting as strong barriers to continued dispersal (such as physical barriers, e.g. dams, culverts). The Westley and Fleming (2011) study also examined rate of invasion in Newfoundland of brown trout. They were found to have an average invasion rate of 4km per year so far, so they have spread roughly 500km from source in almost 130 years. This rate is considered slow when compared with other salmonid species such as chinook salmon (*Oncorhynchus tshawytscha)* in South America (54km per year, Correa and Gross, 2008) and New Zealand (13km per year, Unwin and Quinn, 1993), but is consistent with recorded rates of brown trout spread in the Kerguelen Islands (Ayllon *et al.*, 2006).

Invasive species cause economic and environmental damage worldwide and the control and management of these species is complicated by the lack of understanding of their methods and routes of spread. Population genetics theory and methods provide a number of possible aids in understanding these processes. As stated above, brown trout, *Salmo trutta* were first introduced to Newfoundland, in 1883 and have spread and established successful invasive populations in the intervening 130 years.

3.1.2 Main Hypotheses of this Chapter

The hypotheses examined by this study concern the genetic make-up of the invasive brown trout populations on the Avalon Peninsula. Firstly, it was hypothesised that the study would provide genetic confirmation of the source of the brown trout introduction, to clarify and confirm historical records and to verify the theories presented by Westley and Fleming (2011) and Westley *et al.* (2012), based on ecological investigations, in relation to the direction and means of invasion; that is mainly that fish migrated in a stepping stone pattern by means of anadromous migration. Verifying records is important, as certain historical data on invasive species have been shown to be incomplete or erroneous, in other incidences of successful invasions. Knowledge on the details of the invasion is also critical when trying to control invasive species, so the ability of genetic markers to confirm or refute data that would otherwise be called into question, is highly valued.

It was also hypothesised that populations would have diverged genetically from the source population over the course of the last 130 years since introduction and that the predicted lower genetic diversity in these new populations could impact on the species' ability to colonise new habitats at increasing geographic distance for the original site of invasion.

3.2 Materials and Methods

3.2.1 Site description: The Avalon Peninsula, Newfoundland, Canada

The island of Newfoundland is located in the northwest Atlantic off the east coast of the North America. It is part of the Canadian province of Newfoundland and Labrador (Figure 1). Newfoundland is separated from mainland Canada by the Straits of Belle Isle. The island has an area of 108,860km^2 and a circumference of approximately 12,000 km. A large part of the island of Newfoundland is an extension of the North American Appalachian mountain system with the eastern part of the island, including the Avalon Peninsula, made up mainly of folded sedimentary rocks with some intrusions of igneous rock (Bell and Liverman, 2008). Newfoundland Island is considered to be a cool summer subtype of a humid continental climate, greatly influenced by the sea, since no part of the island is more than 100 km from the ocean.

The Avalon Peninsula, on the eastern side of the island is the most populous portion of the island, with the capital, St. John's, located on the east coast of the peninsula (Figure 1). The city of St. John's is approximately 350km from the main portion of Newfoundland, travelling in a northerly direction, while travelling south along the coast of the Avalon Peninsula, there is roughly 420km of coast before the main part of insular Newfoundland is reached.

Insular Newfoundland was completely covered by ice during the last glacial maximum. The lack of soil on most parts of the island is a result of the scouring effect of glaciers during this time (Westley and Fleming, 2011).The island exhibits a typical glaciated topography, with shallow soil and bedrock scored by glaciers responsible for the numerous lakes and short, swift flowing rivers found across Newfoundland Island (Bell and Liverman, 2008). Rivers in Newfoundland tend to be unproductive and nutrient poor. The only lake examined in this study is Windsor Lake, a naturally land-locked oligotrophic water body located in the environs of St. John's (Westley *et al.*, 2013). Native salmonid species are restricted to the brook

trout *Salvelinus fontinalis* and the Atlantic salmon, *Salmo salar*. Native here is defined as species that naturally recolonised previously glaciated areas after the last glacial maximum.

3.2.2 Sample collection

The history of the introduction of brown trout to Newfoundland is discussed in Section 3.1.1 of this chapter. Brown trout were introduced over several years in the St. John's area, with 156,000 fry estimated to have been released into the local systems between 1883 and 1906 as a public works effort to enhance the fisheries of the region (Westley and Fleming, 2011).

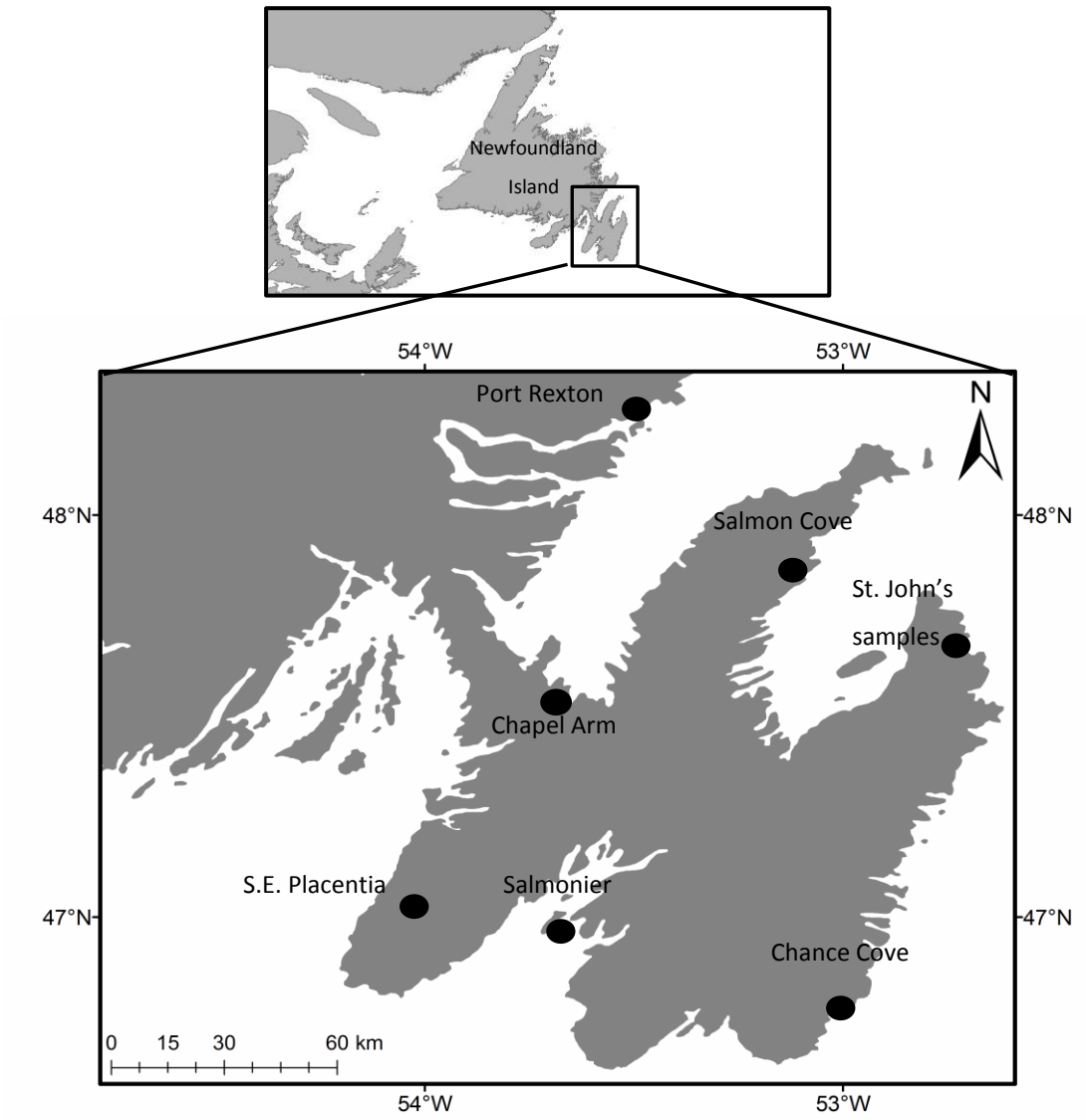


Figure 1: Map showing location and names of sampling sites around the Avalon Peninsula, Newfoundland, marked with black dots.

Sites were sampled for genetic analysis throughout the Avalon Peninsula, , during the summers of 2008 and 2010 (Table 1, Figure 1). A single pass electrofishing method was used by experienced operators (Smith Root LR-24 electrofisher) to collect juvenile brown trout (*Salmo trutta*). Where possible, 300-400m of river was sampled to decrease the possibility of collecting 0+ fish from relatively few families. A minimum of 50 fish were sampled at each site in each summer. Where a sample was not collected in 2008, two age classes were sampled in 2010, to allow for the testing of temporal stability across all sites. Age classes were distinguished in the field by body length and later validated by scale reading. Sites were selected based on the known presence of brown trout, as this species are not present in all catchments of the Avalon Peninsula (Department of Fisheries and Oceans Angling Guide 2008) and on spatial distance from the known site of first introduction in the Rennies River catchment in St. John's.

Table 1: Details of the sampling locations on the Avalon Peninsula, Newfoundland used in the study. At some sites, two age classes were collected in one year, while in other cases samples of were collected on two different years, N=50 for each sample.

Region	Sampling site	Latitude	Longitude	Sampling Years	Age Classes
St. John's	Windsor-Parkers Brook	47°36'6.10"N	52°46'46.01"W	2008, 2010	0+
St. John's	Windsor-Middle Rocky Br.	47°35'32.07"N	52°47'48.16"W	2010	0+, 1 +
St. John's	Rennie's River	47°34'40.46"N	52°42'57.34"W	2008, 2010	0+
St. John's	Virginia River	47°35'18.66"N	52°41'26.92"W	2008, 2010	1+
St. John's	Waterford River	47°31'30.63"N	52°44'58.95"W	2008, 2010	0+, 1+
North Avalon	Salmon Cove River	47°46'54.47"N	53°10'11.60"W	2008, 2010	0+
North Avalon	Chapel Arm River	47°30'45.99"N	53°40'43.64"W	2010	0+, 1+
North Avalon	Port Rexton	48°23'48.41"N	53°19'43.62"W	2008, 2010	0+
South Avalon	Chance Cove Brook	46°46'18.90"N	53° 0'59.04"W	2008, 2010	0+
South Avalon	Salmonier River	47°10'25.87"N	53°39'46.92"W	2008, 2010	0+, 1+
South Avalon	SE Placentia River	47°13'20.70"N	53°53'38.43"W	2008, 2010	0+

It was assumed that the sites furthest from the source of introduction (St. John's) were the most recent introductions. This assumption is mainly backed up by data from surveys by the Department of Fisheries and Oceans (DFO) (DFO, 2010 and Westley and Fleming, 2011). Sites were sampled in St. John's itself, from the landlocked lake where the fish were initially introduced in 1883, two rivers within the catchment that was the point of the main introduction between 1883 to 1906, a

second neighbouring catchment within the city environs and then three sites each to the north and to the south of the Avalon Peninsula at progressively greater geographic distances (See Figure 1 for a map of sites and Table 1 for details of the sampling locations used).

Samples of juvenile brown trout were killed by overdosing in clove oil and a tissue sample consisting of muscle or caudal fin was taken, which was preserved in 98% ethanol in an individually labelled Eppendorf 1.5ml vial. Samples were shipped back from Newfoundland to the Population Genetics Laboratory, School of Biological, Earth & Environmental Sciences, University College Cork (UCC) at the end of the sampling season in 2010. As stated above, a minimum of 50 trout samples was taken each year over two years (or depending on site, two age classes in 2010) for 12 sites, resulting in a total of 1,200 individual samples from Newfoundland. All sample sites are presented in the same sequence throughout this chapter. Sample sites are shown in terms of one of three general regions: St. Johns, North Avalon and South Avalon. These groups are then presented in terms of geographic distance from the site of first introduction in St. Johns (Windsor Lake), e.g. North Avalon samples: Salmon Cove is closest to St. Johns, with Chapel Arm intermediate and Port Rexton most distant (Figure 1). Note that although Port Rexton is located off the Avalon Peninsula, on the Bonavista Peninsula, it is referred to as being part of the North Avalon group in the present study. Predicted rate of invasion, based on information collated from previous work and local angling knowledge, was used as the basis of the sampling strategy employed here. It was expected that, after initial introductions in the vicinity of St. John's that brown trout migrated along the coast of the Avalon Peninsula in both a northerly and southerly direction.

As stated above, historical records show the vast majority of the introduced brown trout were imported from the Howietoun hatchery, Stirling, Scotland throughout the 1890's. While excellent records have been kept of the transport of brown trout ova (University of Stirling archive), there are no tissue samples remaining. Dr. Eric Verspoor of Marine Scotland, Pitlochry kindly supplied a sample of fifty modern scale sets from Loch Leven, the known wild source of most Howietoun broodstock. These samples were collected by anglers from the Loch Leven fishery during June and July 2001. The Howietoun hatchery also allowed access to its modern juvenile

brown trout population, enabling collection of a sample from both the 0+ and 2+ hatchery cohorts in 2011. Collection of samples was undertaken in conjunction with an in-depth ecological study of brown trout on the Avalon Peninsula, conducted by Peter Westley and Ian Fleming, of Memorial University, Newfoundland.

Certain documentary evidence suggested a possible introduction involving brown trout derived from the German "von Behr" strain, which are believed to be derived from brown trout collected from tributaries of the Elbe in the vicinity of Hamburg in the 1880's and 1890's (Westley and Fleming, 2011). Contemporary German *S. trutta* samples, previously used in an experiment to examine invasion of brown trout in South America (Valiente *et al.*, 2011) were kindly supplied by E. Vasquez and A. Valiente (University of Oviedo, Spain) from several sites around Germany for comparison.

3.2.3 Molecular analysis

DNA was extracted from tissue samples, using the Puregene DNA extraction procedure (Qiagen Ltd), a salting-out method which gives high molecular weight, archival quality DNA. Quantification of extracted DNA was carried out using a Nanodrop ND-1000, and quality was assessed by running a subsample from each group of 96 individual samples on a 1.5% Agarose gel. Amplification reactions were carried out with a total reaction volume of 3.5µl for 21 microsatellite loci (20 neutral markers and MHC1) using two multiplex panels. The protocols for the multiplex panels were provided by the Population Genetics Laboratory in Queens University, Belfast. These panels were optimised for use as part of the Celtic Sea Trout project (Keenan *et al.*, 2013). Details of loci used are given in Table 2, with the panel recipes given in Appendix I.

PCR consisted of 1 µl DNA extract (concentration ranging from 4 -10ng), forward and reverse primers at a concentration of 20 pM/µl and PPP Master Mix (2x concentrated, Top Bio Ltd.) made up of 150 mM Tris-HCl, pH 8.8 (at 25° C), 40 mM $(NH_4)_2SO_4$, 0.02% Tween 20, 5 mM $MgCl_2$, 400 µM dATP, 400 µM dCTP, 400 µM dGTP, 400 µM dTTP and 100 U/ml of Taq DNA polymerase. PCRs were

carried out in 96 well microtitre plates. Each sample was overlaid with 10 µl of mineral oil to prevent evaporation. PCR amplifications were carried out using a Techne TC-Plus thermocycler. The PCR cycle for Panel One followed an initial denaturation period of 95°C for 15 minutes, 25 cycles of 94°C for 30 seconds, 57°C for 1 minute 30 seconds, and 72°C for 1 minute with a final cycle of 60°C for 30 minutes. The PCR cycle for Panel 2 also used an initial denaturation period of 95°C for 15 minutes, followed by five cycles of 94°C for 30 seconds, 55°C for 1 minute 30 seconds, and 72°C for 1 minute and then twenty cycles of 94°C for 30 seconds, 57°C for one minute 30 seconds, and 72°C for 1 minute, with a final cycle of 60°C for 30 minutes.

Table 2: Panel information showing loci used, primer sequences (with ABI-labelled primer) and original literature references. Adapted from Keenan *et al.* (2013).

Locus	Forward Primer	Reverse primer	Reference
Panel One			
Ssa416	FAM-TGACCAACAACAAACGCACAT	gtttCCCACCCATTAACACAACTAT	Cairney *et al.* (2000)
One103	FAM-GGGTACCCACTGACGCTATG	gttTCTGGTACTTCCCTGATGC	Olsen *et al.* (2000)
SsaD48	FAM-GAGCCTGTTCAGAGAAATGAG	gtttCAGAGGTGTTGAGTCAGAGAAG	King *et al.* (2005)
Cocl-Lav-4	VIC-TGGTGTAATGGCTTTTCCTG	gtttGGGAGCAACATTGGACTCTC	Rogers *et al.* (2004)
CA048828	VIC-GAGGGCTTCCCATACAACAA	gtttGTTTAAGCGGTGAGTTGACGAGAG	Vasemagi *et al.* (2005a)
One9µ	NED-CTCTCTTTGGCTCGGGGAATGTT	gtttGCATGTTCTGACAGCCTACAGCT	Schribner *et al. (1996)*
Ssa85	NED-AGGTGGGTCCTCCAAGCTAC	gtttACCCGCTCCTCACTTAATC	O'Reilly *et al.* (1996)
One102-a	NED-GGGATTATTCTTACTTTGGCTGTT	gtttCCTGGTTGGGAATCACTGC	Olsen *et al.* (2000)
One102-b	NED-GGGATTATTCTTACTTTGGCTGTT	gtttCCTGGTTGGGAATCACTGC	Olsen *et al.* (2000)
Ssa406UoS	NED-ACCAACCTGCACATGTCTTCTATG	gtttGCTGCCGCCTGTTGTCTCTTT	Cairney *et al.* (2000)
CA054565	VIC-TCTGTGGTTCCCGATCTTTC	gtttCAACATTTGCCTAGCCCAGA	Vasemagi *et al.* (2005b)
CA053293	PET-TCTCATGGTGAGCAACAAACA	gtttACTCTGGGGCATTCATTCAG	Vasemagi *et al.* (2005a)
Str2QUB	PET-CTGGGGTCCACAGCCTATAA	gtttGAGCTACAACCTGATCCACCA	Keenan *et al.* (2013)
One108	VIC-GTCATACTACTCATTCCACATTA	gtttACACAGTCACCTCAGTCTATTC	Olsen *et al.* (2000)
Panel Two			
Str3QUB	FAM-CTGACCGCTGCACACTAA	gtttGGCTCTAATCGACTGGCAGA	Keenan *et al.* (2013)
CA060177	VIC-CGCTTCCTGGACAAAAATTA	gtttGAGCACACCCATTCTCA	Vasemagi *et al.* (2005b)
Ssa197	VIC-GGGTTGAGTAGGGAGGCTTG	gttTGGCAGGGATTTGACATAAC	O'Reilly *et al.* (1996)
MHCI	PET-AGGAAGGTGCTGAAGAGGAAC	gtttCAATTACCACAAGCCCGCTC	Grimholt *et al.* (2002)
SsaD71	NED-AACGTGAAACATAAATCGATGG	gtTTAAGAATGGGTTGCCTATGAG	King *et al.* (2005)
SaSaTAP2A	gtttGTCCTGATGTTGGCTCCCAGG	NED-GCGGGACACCGTCAGGGCAGT	Grimholt *et al.* (2002)
BG935488	gttTGACCCCACCAAGTTTTTCT	NED-AAACACAGTAAGCCCATCTATTG	Vasemagi *et al.* (2005b)
Ssa410UoS	gtttGGAAAATAATCAATGCTGCTGGTT	PET-CTACAATCTGGACTATCTTCTTCA	Cairney *et al.* (2000)

After PCR, the amplified fragments were diluted to one tenth of their strength with double-distilled water. 1 µl of this diluted solution was then added to 9 µl of HiDi formamide (Life Technologies) which was mixed with GeneScan 600-LIZ size

standard (Life Technologies). Diluted, prepared samples of PCR product were shipped from the Population Genetics Laboratory in UCC to Queens University, Belfast, where they were denatured and alleles were resolved on an ABI 3730xl DNA Analyser (96 capillary, ABI Applied Biosystems, Ltd.). Fragment length was determined using GeneScan 600-LIZ size standard and alleles were scored using Genemarker v1.6 (Applied Biosystems). To ensure the accuracy of genotype data, two control genotypes were selected at random from the collected samples and were included on each 96-well plate, with two control samples and a water blank on each plate. Control samples were used to calibrate each plate and also to confirm orientation of samples on the plate itself. Genotyping was carried out by one reader to ensure concordance of allele calls and binning consistency.

From the original panel of twenty two loci, it was found that six loci amplified poorly for this particular set of samples and were therefore unreliable when it came to scoring (*CAO54565, CAO53293, One103, One108, SsaD48* and *BG9335488*). *MHC 1* was not used for this part of the analysis as it is not considered a neutral marker (de Eyto *et al.,* 2011). The locus *One102* was found to be co-amplifying using the primer set for the "a" locus and was therefore separated into *One102-a* and *One102-b*. The finalised dataset therefore contained sixteen loci which were used for data analysis: *Ssa416UoS, Cocl-Lav-4, One9μASC, CAO48828, Ssa85, One102-a, One102-b, Ssa406Uos, CAO54565, Str2QUB, Str3QUB, CAO60177, Ssa197, SsaD71, SaSaTAP2A* and *Ssa410UoS*.

For the German samples, due to our inability to extract high quality DNA from most samples (which had begun to degrade), comparison was only possible with the Scottish and Newfoundland samples at seven loci. These loci were *Ssa416UoS, Cocl-Lav-4, Ssa85, One102-a, CAO54565, Str3QUB,* and *Ssa197*.

3.2.4 Statistical analysis

A total of 1139 individual fish were successfully analysed from 11 sites throughout the Avalon Peninsula (61 samples were excluded as genetic material could not be recovered, mainly due to very small sample size, or were of poor quality), along with

contemporary samples from Loch Leven and the Howietoun hatchery. The data were firstly checked for missing values. Multilocus genotypes that were less than 85% complete were discarded (assigned alleles present for at least 14 out of 16 loci). Scored alleles were checked for potential genotyping errors and inconsistencies using Microsat Toolkit (Parks, 2001). Allele number and allelic richness were calculated using the computer programme FSTAT v2.9.3 (Goudet, 2001). The percentage number of alleles observed in each sample, in relation to the total number of alleles observed among all samples, was calculated (allele frequency). Observed (H_O) and expected (H_E) heterozygosities within populations for all loci and all locations were estimated using the programme GENEPOP v4.2 (Raymond and Rousset, 1995, Rousset, 2008). Deviations from Hardy-Weinberg Equilibrium were estimated using MCMC (Markov Chain Monte Carlo) with 10,000 dememorisation steps, 1,000 batches and 10,000 iterations per batch using GENEPOP. Significance values were adjusted for multiple pairwise tests using Bonferroni correction (Rice, 1989). GENEPOP was used to test for the occurrence of genetic linkage disequilibrium for each pair of loci in each sample. Tests for family effects between temporal samples from the same site were carried out using COLONY v2.0.4.4 (Jones and Wang, 2009). All loci were tested for evidence of natural selection pressures using LOSITAN 2 selection detection workbench, using the F_{ST} outlier approach (Beaumont *et al.*, 1996, Antao *et al.*, 2008) (infinite mutation model, 100,000 simulations). To test for temporal stability between samples from the same location, an exact test using GENEPOP was utilised. The programme Free NA (Chapuis and Estoup, 2007) was used to estimate frequency of null alleles.

To assess levels and patterns of population structuring within the data, a number of approaches were used. First, a non-parametric factorial component analysis (FCA) was carried out using GENETIX v4.05 (Belkhir *et al.*, 2004). In general terms, this analysis partitions individual multi-locus genotypes into a three-dimensional space, allowing for an initial visual assessment of the magnitude of population structuring within the data. Secondly estimates of global and pairwise genetic differentiation were generated using unbiased F_{ST} (Weir and Cockerham, 1984). These values were calculated by accounting for null alleles using FreeNA. Genetic distances between population samples were estimated using POPULATIONS v 1.2.31(Langella, 1999), using Nei's *et al*. (1983) D_A method. Resulting pairwise genetic distances were used

to construct an un-rooted neighbour joining phylogenetic tree. Robustness for tree nodes was obtained through bootstrapping (1,000) individuals over samples. The resulting tree with bootstrap support values was plotted using the programme DENDROSCOPE v3 (Huson and Scornavacca, 2012) which was used to visualise an unrooted neighbour joining tree.

Possible correlations between geography and genetic differentiation between populations (i.e. isolation by distance) were tested for using a Mantel test as implemented in the Isolation by Distance Web Service v 3.23 (IBDWS, Jensen *et al.,* 2005) based on a matrix of genetic distance (measured as F_{ST}) and geographic distance (measured as most likely path of migration by sea) by means of a Mantel test (10,000 iterations).

Bayesian structure analysis was used to estimate the best number of evolutionary significant units (i.e. populations) explaining the data (STRUCTURE v2.3.4, Pritchard *et al.,* 2000, Hubitz *et al.,* 2009). The Bayesian clustering algorithm implemented into the programme STRUCTURE assigns individuals to specific genetic groups (*K*), without "*a priori*" information of geographical location. The number of clusters present in the dataset was inferred using the method of Evanno *et al.* (2005). STRUCTURE analysis was carried out following hierarchical approach, as described by Perrier *et al.* (2011) to allow for the presence of both high and low levels of genetic divergence among samples. Thus, once the best estimate of *K* was identified following the first run of STRUCTURE, each *K* group was subsequently independently analysed again within the STRUCTURE framework. This hierarchical approach was iteratively used until no further genetic structuring was evident from the data. For the STRUCTURE runs, a model assuming admixture with correlated allele frequencies and no prior population information was used. In each case, 20 iterations for each K value ranging from one to 20 were carried out using 100,000 burn-in replicates followed by a Markov Chain Monte Carlo length of 100,000. The program CLUMPP v 1.1.2 (Jakobsson & Rosenberg 2007) was used to consolidate membership coefficients for the 20 iterations for each K estimate. Given the large data set, the "Greedy" algorithm within CLUMPP was used, with 1,000 repeats. Input files for the CLUMPP analysis were assembled using Structure Harvester software utility (Earl & Vonholdt 2012).

The approximate Bayesian computation (ABC) approach, implemented in the DIYABC software (Cornuet *et al.* 2010) was also used to test for alternative scenarios related to the origin(s) and/or possible colonisation patterns of *S. trutta* in Newfoundland outside St. John's. Three different colonisation scenarios were considered. For Scenario 1, *S. trutta* populations from the northern and southern groups are assumed to have been established from early natural sea trout migrants (colonisers) which found their way to sea from an ancestral St. John's population. Under this natural migration process, the pattern of colonisation/invasion is thought to have followed a typical isolation by distance and stepping stone model (i.e. natural gradual and independent north and south seaward migration from St. John's). Scenario 2 is similar to Scenario 1, but with an important difference. Under this scenario, the *S. trutta* population inhabiting the Salmonier River is derived from migrants directly from St. Johns, possibly introduced by humans. In Scenario 3, populations comprising the northern and southern groups were established directly from migrants (colonisers) derived from an ancestral St. John's population; possibly introduced by humans. That is, under Scenario 3, the ancestral St. John's population is the direct source of migrants for contemporary populations representing both the northern and southern groups. Prior distributions for demographic parameters were as follows: Uniform [10; 10000] for effective population sizes (similar for all populations) and Uniform [1; 10000] for *T3*, *T2* and *T1*, with $T3 < T2 < T1$. All microsatellites were included in the analysis and the default parameters of the program for microsatellites were used for mutation. For each scenario, 36×10^6 datasets were simulated prior to evaluation of the models, following the guidelines provided in user manual. Thus, for model comparison, the posterior probabilities for each scenario were estimated using logistic regression (Cornuet *et al*. 2008), and these were then used to estimate type I and II errors in the choice of each scenario.

3.3 Results

No examples were found in this study of any misidentified collected samples (showing evidence of being either Atlantic salmon (*Salmo salar)* or brook trout (*Salvelinus fontinalis),* (which cohabit in all sites sampled). Also, there were no examples of hybrids (either Atlantic salmon x brown trout or brown trout x brook trout).

3.3.1 Intra sample variation

A total of 1139 *S. trutta* from thirteen sampling areas were successfully genotyped for 16 loci. The sites sampled were made up of two Scottish sites (indicative of presumed origin), six sites within the city environs of St. John's, three sites on the north coast of the Avalon Peninsula and a further three sites along the south coast of the Avalon Peninsula (Figure 1, Table 3). These are arbitrarily defined as Scottish, St. John's, North Avalon and South Avalon clusters respectively, and subsequent genetic analyses (see below) confirms this arrangement.

There was no evidence of linkage disequilibrium between loci, nor was there evidence of natural selection acting on any loci. No problems with consistent genotyping errors were detected using Micro-Checker, although there was some evidence of null alleles. Observed (H_O) and expected heterozygosity (H_E) are presented in Appendix II (summarised in Table 4), as are p-values from tests of adherence to Hardy-Weinberg expectations. Values of HWE were corrected for significance using a Bonferroni correction. There were no consistent departures from Hardy Weinberg expectations at any particular locus or sample with the exception of the sample from Windsor Lake-Parkers Brook which did not adhere to Hardy-Weinberg expectations for six out of 16 loci, with three loci showing excess heterozygosity, and three loci showing heterozygote deficiencies.

Summary statistics across all loci from all thirteen sampling sites are provided in Appendix II and are summarised in Table 4 with allele frequencies being presented in Appendix III. Testing for family effects, using COLONY software, showed no evidence of over-representation of family groups within any samples. Allelic

richness (A_R) and expected heterozygosity (H_E) were used to indicate within population genetic variability (Table 4 summary, Appendix II).

Table 3: Details of the sampling locations on the Avalon Peninsula, Newfoundland and from the putative Scottish sources of origin used in the study, showing year of collection, age class and number of samples genotyped.

Region	Sampling site	Sample	N	Sample	N	Total
Scotland	Loch Leven	1990's	38			38
Scotland	Howietoun	2010 2+	32	2010 0+	36	68
St. John's	Windsor-Parkers Brook	2008 0+	44	2010 0+	46	90
St. John's	Windsor-Middle Rocky Br.	2010 0+	49	2010 1+	46	95
St. John's	Rennie's River	2008 0+	46	2010 0+	50	96
St. John's	Virginia River	2008 1+	47	2010 0+	39	86
St. John's	Waterford River	2008 1+	47	2010 0+	49	96
North Avalon	Salmon Cove River	2008 0+	49	2010 0+	50	99
North Avalon	Chapel Arm River	2010 0+	47	2010 1+	51	98
North Avalon	Port Rexton	2008 0+	40	2010 0+	48	88
South Avalon	Chance Cove Brook	2008 0+	49	2010 0+	49	98
South Avalon	Salmonier River	2010 0+	45	2010 0+	47	92
South Avalon	SE Placentia River	2008 0+	49	2010 0+	46	95

3.3.2 Inter sample variation (temporal)

Because some evidence of null alleles had been demonstrated, F_{ST} values were estimated using the programme FreeNA which corrects for the effects of null alleles. No statistically significant differences were found using exact tests between temporal samples at any of the sites studied (Appendix IV). This indicated temporal stability of allele frequencies of the invasive populations at these sites between the years sampled. As a result, temporal samples were combined and all analyses from here onwards were carried out on combined temporal samples from each site.

Table 4 - Summary statistics for *S. trutta* samples screened for 16 microsatellite loci: ***N*** = number of individuals screened per sample; ***A*** (***T%***)= number of alleles (% observed in sample in relation to total observed among all samples); H_o = observed heterozygosity; H_e = expected heterozygosity (Nei, 1987); ***A*r** = Allelic richness following the rarefaction method (Petit et al., 1998); **HWE** = *P* values of exact tests for non-conformance to Hardy-Weinberg Expectations (Guo & Thompson, 1992). Significant values after Bonferroni correction given in bold. Averages values per loci and per population sample are also provided.

Region	Catchment	Site		Average	Total NA	No. loci out of HWE	% loci out of HWE
Scotland		Lough Leven Wild	N	37			
			A (%T)	10 (48.6%)	160		
			H_O	1			
			H_E	1			
			HWE			0	0
Scotland		Howietoun 2011 Hatchery	N	66			
			A (%T)	9.8 (47.4%)	156		
			H_O	1			
			H_E	1			
			HWE-R			2	12
St. John's	Windsor Lake	Parker's Brook	N	87			
			A (%T)	8.4 (40.7%)	134		
			H_O	1			
			H_E	1			
			HWE			6	37
St. John's	Windsor Lake	Windsor Brook 2	N	94			
			A (%T)	8.2 (39.8%)	131		
			H_O	1			
			H_E	1			
			HWE			2	12
St. John's	Rennies	Rennies River	N	96			
			A (%T)	11.6 (56.2%)	185		
			H_O	1			
			H_E	1			
			HWE			2	12
St. John's	Rennies	Virginia River	N	84			
			A (%T)	10.3 (50.2%)	165		
			H_O	1			
			H_E	1			
			HWE			0	0
St. John's	Waterford	Waterford River	N	95			
			A (%T)	8.6 (41.9%)	138		
			H_O	1			
			H_E	1			
			HWE			2	12
North	Salmon Cove	Salmon Cove	N	99			
			A (%T)	8.6 (41.9%)	138		
			H_O	1			
			H_E	1			
			HWE			1	6
North	Chapel Arm	Chapel Arm	N	97			
			A (%T)	6.4 (31%)	102		
			H_O	1			
			H_E	1			
			HWE			0	0
North	Port Rexton	Port Rexton	N	86			
			A (%T)	5.8 (28.3%)	93		
			H_O	1			
			H_E	1			
			HWE			2	12
South	Chance Cove	Chance Cove	N	97			
			A (%T)	7.7 (37.4%)	123		
			H_O	1			
			H_E	1			
			HWE			3	19
South	Salmonier	No Name Brook	N	92			
			A (%T)	6.6 (31.9%)	105		
			H_O	1			
			H_E	1			
			HWE			2	12
South	South East Placentia	South East Placentia	N	94			
			A (%T)	5.2 (25.2%)	83		
			H_O	1			
			H_E	1			
			HWE			1	6

3.3.3 Inter sample variation (spatial)

Provenance of "source" population

The contemporary sample (1990's) collected from Loch Leven shows a close relationship (in terms of allele frequencies, Appendix III) with the current sample collected in the Howietoun hatchery, suggesting the hatchery line is descended from wild Loch Leven stock, as was expected from hatchery records. The same subset of alleles appears to occur in Newfoundland samples collected from in and around the St. John's region (Figure 1), as are found in the two Scottish samples (Loch Leven and Howietoun, Appendix II and III), indicating the main invasion event was indeed made up of brown trout derived from the Loch Leven strain, as reported previously (Maitland, 1887, Westley and Fleming, 2011). However, there are some examples in Appendix III of alleles being represented in the St. John's "source" and North and South Avalon "invasive" populations which are not present in either of the two donor populations.

Genetics of the invasion

Appendix II shows a reduction of allelic richness and total number of alleles present in the various "invasive" populations as you move away, both to the north and south of the Avalon Peninsula, from the assumed "source" populations in the St. John's region, indicating the possibility of a stepping stone invasion model. Analysis of putative "source" populations (St. John's group) and "invasive" populations North Avalon and South Avalon groups) showed similar trends as those discussed above in population differentiation across different types of analysis (see below).

F_{ST} among all samples was 0.091, with F_{ST} per locus ranging from 0.069 to 0.110. Pairwise population F_{ST} (Table 5) values ranged from 0.009 to 0.164 with 95% confidence intervals all showing positive values, indicating statistically significant differences between sites, that is, all sites were genetically distinct from each other. The table of pairwise F_{ST} values shows lower values within geographical clusters (St. John's, North Avalon and South Avalon) than between clusters. There is also some evidence of different alleles being represented in the northern Avalon and southern Avalon samples (Appendix III), which would have been geographically

separated from each other since they migrated away from the source populations (though this result might also be due to sampling error). This indicates a lack of further human intervention (at least successfully) in the invasion process. Looking at samples as the distance increases from the suggested original introduction in the St. John's area to the north and south, there does seem to be a general trend towards a lower number of alleles appearing in some loci (Appendix III, e.g. *Ssa416, Str2QUB, Ssa406UOS),* indicating a stepping stone model of colonisation.

Table 5: Matrix of pairwise F_{ST} values (corrected for effect of null alleles, Chapuis & Estoup, 2007) between sampling sites across all loci, on the top portion of the matrix, and distances (km) between sampling sites on the bottom portion. Distances were estimated by measuring the most likely route of migration by sea for *S. trutta*. Text in italics indicates sites sampled in the St. John's area, those with a thick underline indicate sites from the North Avalon and double underline indicates South Avalon sites.

	Scotland	Scotland	*St. John's*	*St. John's*	*St. John's*	*St. John's*	*St. John's*	N. Avalon	N. Avalon	N. Avalon	S. Avalon	S. Avalon	S. Avalon
	Lough Leven	Howietoun	*Parkers Brook*	*Mid. Rocky Brook*	*Rennies River*	*Virginia River*	*Waterford River*	Salmon Cove	Chapel Arm	Port Rexton	C. Cove Brook	Salmonier River	SE Placentia River
L. Leven		0.037	0.031	0.028	0.029	0.032	0.048	0.047	0.094	0.114	0.075	0.077	0.101
Howietoun	-		0.055	0.055	0.050	0.059	0.066	0.083	0.129	0.152	0.098	0.092	0.136
Parkers Br.	-	-		0.009	0.051	0.054	0.068	0.072	0.105	0.113	0.082	0.074	0.113
Mid. R. Br.	-	-	1.5		0.047	0.044	0.058	0.066	0.110	0.123	0.077	0.081	0.117
Rennies R.	-	-	3.5	5.0		0.033	0.052	0.065	0.116	0.127	0.091	0.096	0.108
Virginia R.	-	-	4.0	5.0	2.0		0.061	0.067	0.119	0.135	0.108	0.097	0.130
Waterford R.	-	-	10.0	11.0	6.0	6.0		0.067	0.115	0.155	0.113	0.086	0.133
Salmon Cove	-	-	87.0	88.0	92.0	92.0	98.0		0.069	0.105	0.100	0.077	0.128
Chapel Arm	-	-	245.0	246.0	250.0	250.0	256.0	158.0		0.107	0.139	0.116	0.146
Port Rexton	-	-	150.0	151.0	155.0	155.0	161.0	63.0	95.0		0.164	0.117	0.164
C. Cove Br.	-	-	144.0	145.0	140.0	140.0	134.0	232.0	390.0	295.0		0.114	0.073
Salmonier R.	-	-	260.0	261.5	251.0	249.0	250.0	350.0	513.0	446.0	136.0		0.109
SE Plac. R.	-	-	425.0	426.0	421.0	421.0	415.0	513.0	671.0	576.0	281.0	137.0	

Isolation by distance analysis (Mantel test) showed a strong positive relationship between geographical distance (km) and genetic differentiation (F_{ST}) when looking at the Newfoundland sampling sites (r = 0.623, p = 0.001, Figure 2).

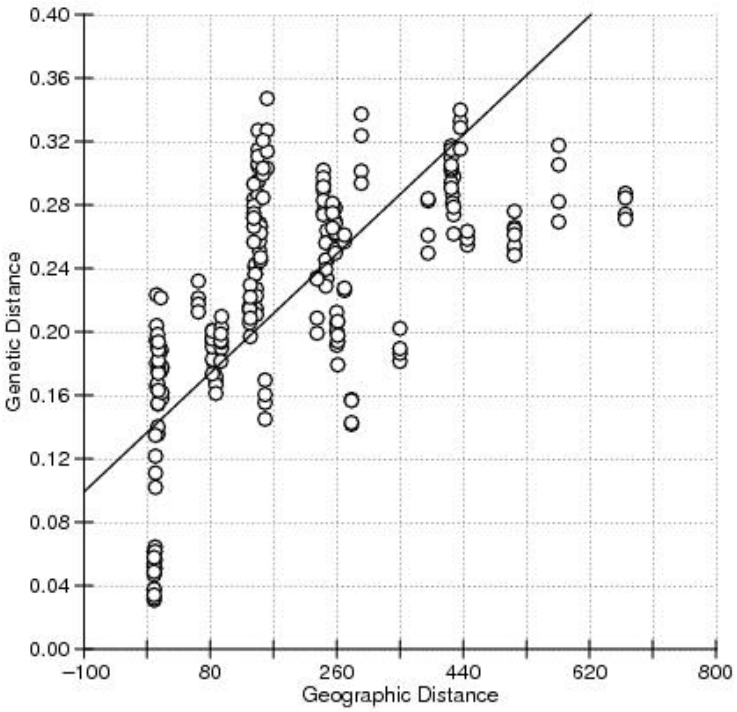


Figure 2: Output from isolation by distance analysis (Mantel test) giving the relationship between geographic (km) and genetic distance (F_{ST}) for Newfoundland sampling sites (r = 0.623, p = 0.001).

Factorial component analysis (FCA) showed strong evidence of the arbitrary clustering suggested above (Figure 3). Figure 3 shows the donor populations (Loch Leven and Howietoun, and the two samples from Windsor Lake) clustering together towards the centre of the plot. Windsor Lake is landlocked and is recorded as the first site in the city of St. John's to be stocked with Loch Leven brown trout. All other source populations from the St. John's region cluster together. The North Avalon and South Avalon groupings also occur as distinct clusters in this analysis (Figure 3).

An unrooted neighbour-joining dendrogram, based on Nei's D_A, shows similar patterns of differentiation as those observed in previous analyses (Figures 2 and 3). Separate temporal samples were used in this analysis and general trends followed those seen in previous analyses, that is, all temporal samples group together. The Scottish samples were less differentiated from the samples collected in the St. Johns region than the samples from the North and South Avalon samples respectively, as was seen previously. This analysis showed less division between the Scottish

samples and the various samples collected in the St. John's region. The samples from North and South Avalon also appeared as distinct groupings in this analysis (Figure 4).

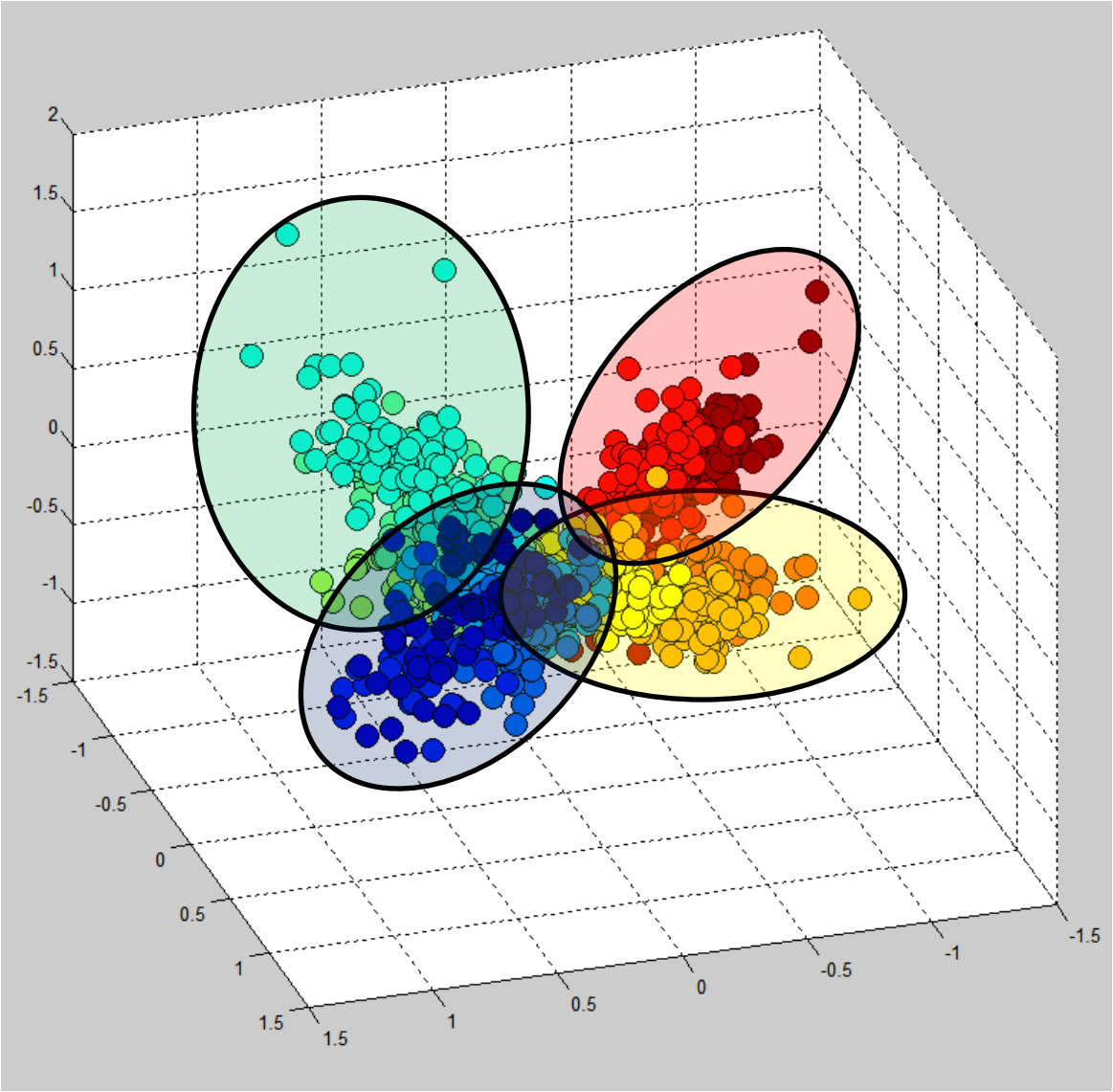


Figure 3: 3D Factorial Component Analysis showing possible population structuring between suggested donor, source and invasive populations. Each spot represents an individual fish. Blue grouping: Loch Leven (not visible, in centre, donor), Howietoun (donor), Windsor Parkers Brook, Windsor Middle Rocky Brook (source). Green grouping: Rennies River, Virginia River, Waterford River (source). Yellow grouping: Salmon Cove, Chapel Arm, Port Rexton (North Avalon, invasive). Red grouping: Chance Cove, Salmonier, South East Placentia (South Avalon, invasive).

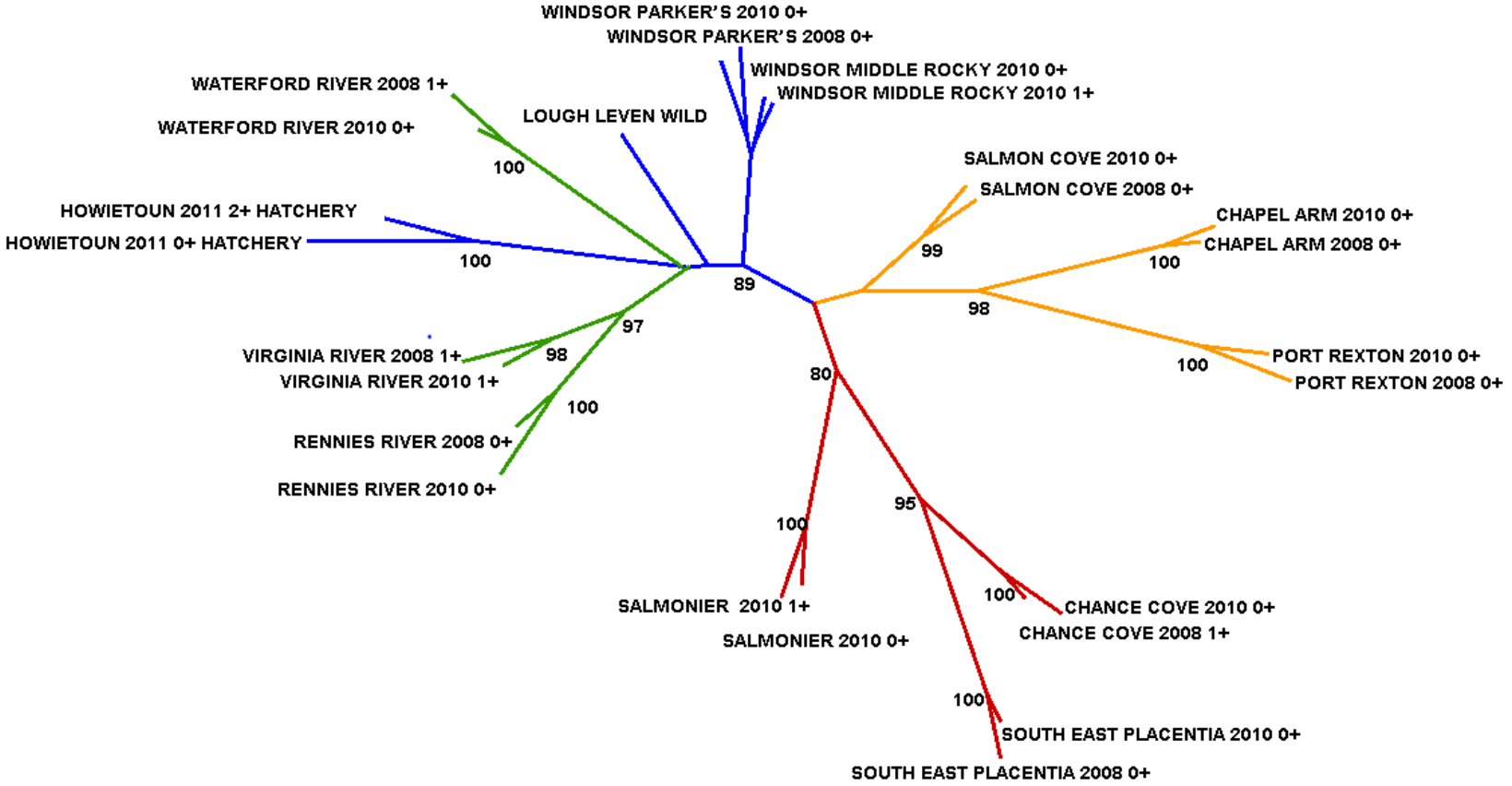


Figure 4: Unrooted neighbour- joining dendrogram based on Nei's D_A (1983) genetic distance illustrating relationship among Scottish and Newfoundland samples (both temporal samples shown at each site), with bootstrap values higher than 80 indicated. Different colours represent major genetic groups as coded in FCA analysis.

Results from the STRUCTURE analysis of individuals show population structuring at two hierarchical levels. Level One, which is all sample sites used in the present study, (Figure 5a), has a suggested K value of four population groupings, based on the peak value of λK (Table 6). When looking at a bar plot of results for this level, the samples from rivers in the St. John's area with free access to the sea (Rennies, Virginia and Waterford, Figure 5a) fall into the same population grouping as the Scottish samples. The samples from the North Avalon region form one population grouping, while samples from the South Avalon region seem to form two distinct invasive population entities, one of which (Salmonier River) appears similar to the St. John's samples sourced from Windsor Lake (Figure 5b). However, analysis shown in Figure 5 seems to suggest that the Salmonier fits well into the South Avalon invasive group.

Table 6: Values of λK (Evanno *et al.*, 2005) giving all suggested values for K number of population groupings at two hierarchical levels. Peak values are highlighted in bold and were used as best estimates of K.

K value	Combined sites	Scotland and St. John's	North Avalon	South Avalon
	K=1 to K=17	K=1 to K=15	K=1 to K=10	K=1 to K=10
1	-	-	-	-
2	3.44	17.26	4.26	**623.38**
3	2.44	1.4	**650.46**	366.82
4	**5.25**	**92.43**	1.03	20.11
5	0.31	38.64	0.33	1.84
6	2.3	1.86	0.18	0.37
7	0.75	1.12	0.57	1.07
8	4.37	0.54	0.56	0.13
9	0.45	0.03	0.58	19.21
10	0.68	0.34	-	-
11	0.16	0.56	-	-
12	0.98	0.37	-	-
13	0.17	0.02	-	-
14	0.34	0.73	-	-
15	4.15	-	-	-
16	0.52	-	-	-
17	-	-	-	-

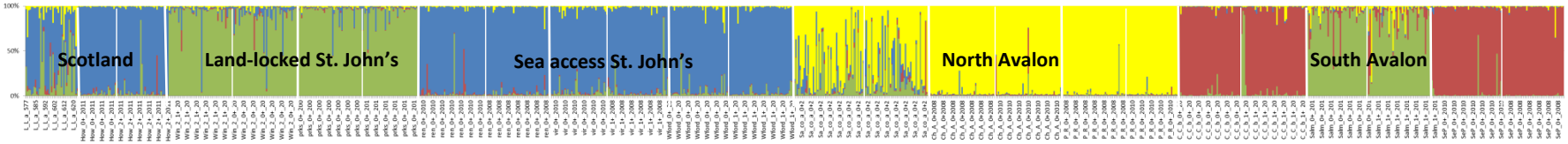
Samples were further divided based on suggested clusters provided by the first level of hierarchical analysis and reanalysed, giving bar plots at hierarchical level two (Figure 5b, c, d). The Scottish population samples were grouped with individuals

sampled in the St. John's region for the second analysis based on their similarities in the first STRUCTURE analysis.

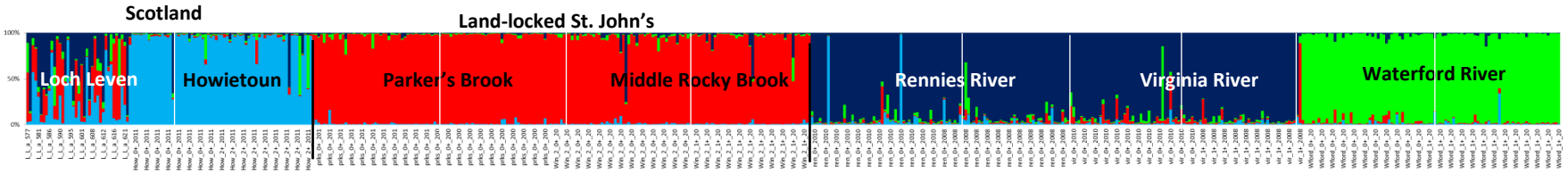
The bar plot of the second analysis (K=4, Figure 5b) shows structuring between the samples in Windsor Lake (Parkers brook and Middle Rocky Brook), the samples from Rennies and Virginia Rivers (same catchment) and the Waterford River, which is a neighbouring catchment to the other two rivers. The Scottish samples from the Howietoun hatchery now clump as a separate population, while the sample from Loch Leven seems to contain a mixture of the genotypes found in the other three aggregations. Loch Leven is recorded as the source population for the majority of *S. trutta* introductions to Newfoundland (Note, however, that the sample in the current study is modern).

Looking at a bar plot of the STRUCTURE results for individuals in the North Avalon region (suggested K of 3, Figure 5c), the clusters suggested by the software seem to be associated with the three rivers, Port Rexton, Chapel Arm and Salmon Cove, with indications of some similarities between sites. This fits well with the geography of the area and expected routes for the species expansion. Results for population structure for the South Avalon region (K=2, Figure 5d) show the same pattern at hierarchical level 2 as they did for the first analysis. Clusters again correspond well to samples. However, individuals from the Salmonier River appear somewhat distinct from trout collected in Chance Cove Brook and the South East Placentia River.

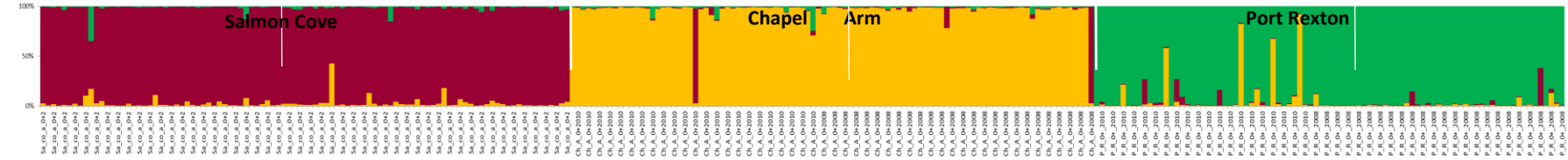
a) Hierarchical level 1: All population samples (K=4)



b) Hierarchical level 2: Scotland and St. John's (K=4)



c) Hierarchical level 2: North Avalon (K=3)



d) Hierarchical level 2: South Avalon (K=2)

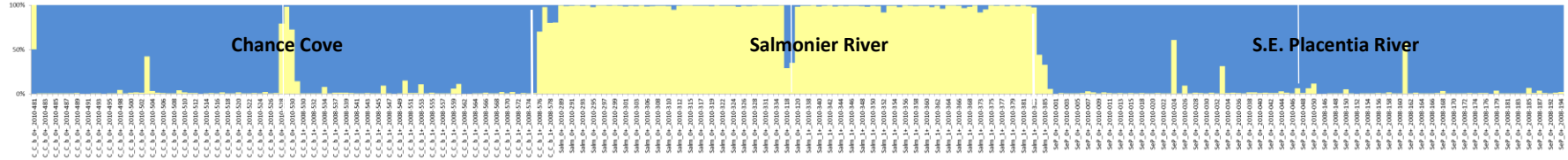


Figure 5: Bar plots of STRUCTURE simulations at two hierarchical levels. Each bar represents an individual fish. Y-axis = proportion of each fish attributed to each cluster, estimated from the colour of the bars. Colours in a) relate to the same groups as shown in Figures 3 and 4, but colours in b), c) and d) are independent.

The results of ABC analysis provide strong support for Scenario 2, as described in Section 3.2.4. That is, excluding Salmonier River, there is good evidence to indicate that *S. trutta* populations along the coast of Newfoundland were established sequentially following a stepping-stone model by natural migrants with initial origin in St. John's. It worth noting that Type I and Type II errors were low for all scenarios (<0.35), clearly indicating that the markers/analysis provided sufficient power to discriminate between models. In summary, the results of this analysis are agreement with previous Mantel test, which indicated a clear correlation between the level of genetic divergence and geographical distance between/among populations. The fact that the *S trutta* population from Salmonier River does not fit to the patterns provides some support to the hypothesis that this particular population was established by introduction of individual directly from St. John's by humans. A graphical representation of the three different scenarios considered in this study is presented in Figure 6.

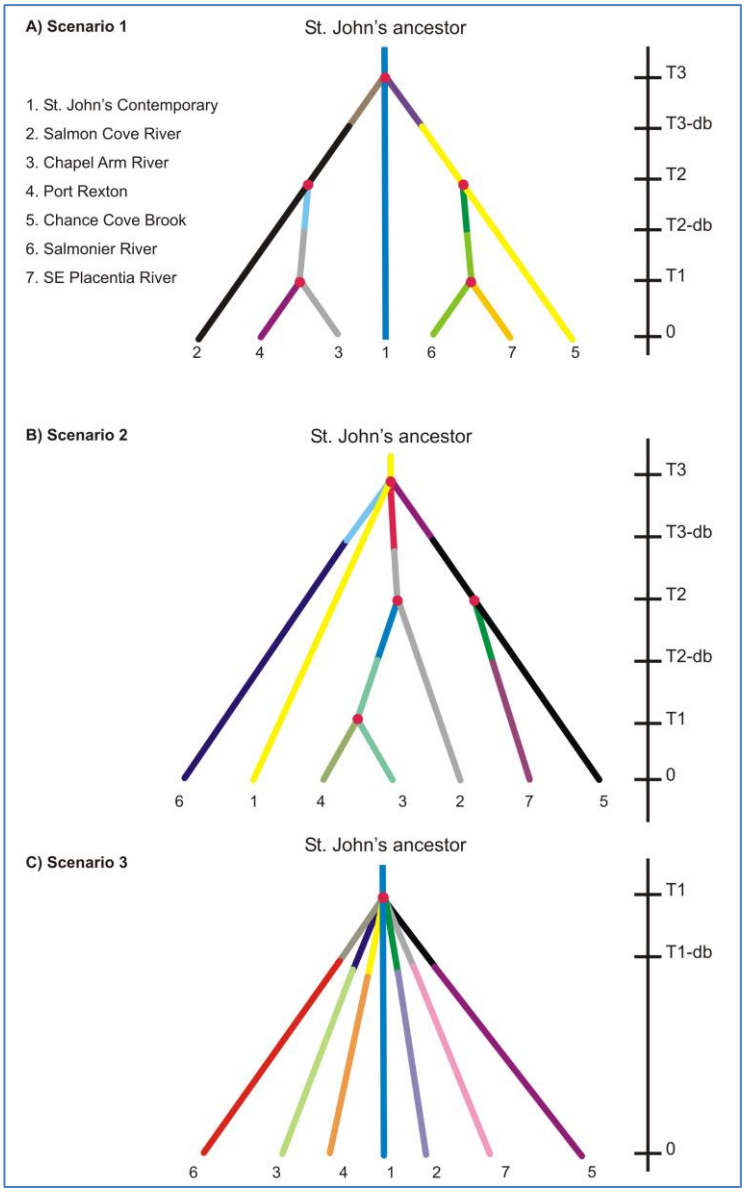


Figure 6. Alternative scenarios (see text for details) related to the origin(s) and/or possible colonisation patterns of *S. trutta* in Newfoundland outside St. John's using the ABC approach, implemented in DIYABC (Cornuet *et al.* 2010). Times of lineage origins are indicated back in time by *T1, T2 and T3,* while *0* indicates present/contemporary time. Here, time is measured in terms of number of generations ('time' in the figure represents evolutionary time). It is important to note that when a new population is formed from individuals migrating from an ancestral population, there is an initial size reduction (noted in the graph by "-db" and distinct colours). This is because new populations generally start with a few immigrants (i.e. founder effect).

As noted above, it has been reported that brown trout eggs sourced from fish originating in Germany and southern England were also imported into Newfoundland from Howietoun hatchery (see Methods section, this chapter). Documentary evidence of the brown trout introduction into Newfoundland indicated

a larger introduction of German strain trout eggs than English strain, although definite numbers are not available (Westley and Fleming, 2011).

Table 7: Main River, tributary and sample sizes for German brown trout samples.

Main River	Tributary	N
Elbe	Lamitz	38
Elbe	Haaraver	19
Elbe	Dreiheirenzrunne	26
Rhine	Ailsbach	9
Rhine	Goldbacl	23
Rhine	Kirnbach	27
Danube	Teisel	28
Danube	Wolenbach	27
Danube	Forrenbach	21
Weser	Grenzbach	22

Supplementary samples of contemporary brown trout sampled from ten rivers around Germany were analysed for seven loci as described above. A dendrogram was created to look at population clustering (Figure 7), which showed that contemporary German trout form a separate grouping so it seems unlikely that there is any German influence in any of the collected Newfoundland samples, so further analysis was not carried out, as it was not felt that comparable, robust results would be obtained due to the poor quality of the German samples. Sites, main source river and sample numbers for the German samples are presented in Table 7.

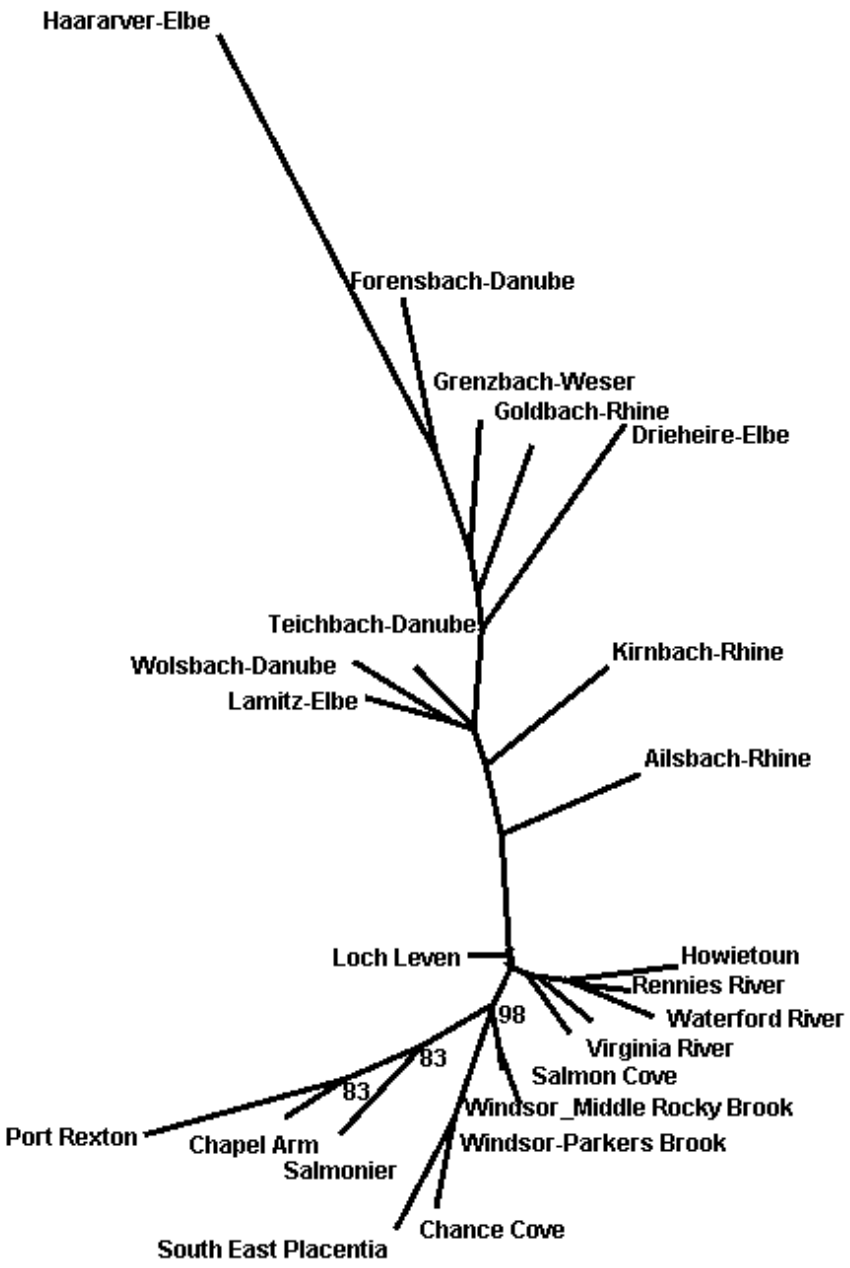


Figure 7: Unrooted neighbour- joining dendrogram based on Nei's D_A (1983) genetic distance illustrating relationship among German, Scottish and Newfoundland samples, with bootstrap values higher than 80 indicated.

3.4 Discussion

This study demonstrates how molecular biological methods can be used to show clearly the routes taken by introduced brown trout as they expanded both to the north and south of the original site of the introduction in St. John's. It is thought this was largely a natural expansion involving anadromous migrations, due to the regular patterns of expansion observed in catchments with no freshwater links and the decline in allelic diversity in populations sampled at increasing geographic distance from the site of introduction.

Genetics of the Invasion

Looking at contemporary brown trout invasive populations on the Avalon Peninsula, our analysis shows three distinct groupings: those sampled around the St. John's region, which are most closely associated with the Scottish samples, the North Avalon samples, and the South Avalon samples. Valiente *et al.* (2007, 2010) and Launey *et al.* (2010) found broadly similar results when studying invasive populations of brown trout introduced into Patagonia and the Kerguelan Islands over similar or shorter time periods. Where our study differs is in the clear pattern shown by the genetic data, both due to the fact that the Scottish invasion seems to have been the only successful introduction (so the data are uncomplicated by introductions from various sites), and the clear pattern shown by the colonisation of new habitats.

The present study found that both the northerly and southerly routes of invasion show evidence of following a classic linear stepping stone model of migration, with allelic diversity decreasing and pairwise F_{ST} values increasing with increasing geographic distance from the initial source of the invasion (Beebee and Rowe, 2004, Allendorf and Luikart, 2007). This could be related to founder effects, where a bottleneck occurs due to a small number of founding individuals, a factor we might expect to see for expansion in brown trout where anadromous individuals are the founders. A negative side to founder effects relates to the resultant reduced genetic variability, with admixture provided by genetic input from various sources considered important for successful introductions (Allendorf and Luikart, 2007), although this idea does not factor in possible effects of out breeding depression.

Previous studies have shown instances where reduction in genetic variability did not constrain the survival or spread of invasives. For example, brown trout populations introduced into Patagonia (Valiente *et al.*, 2007, 2010). The present study shows a high level of genetic variability in the invasive populations in the St. John's regions, and evidence of reduced variability as geographic distance increases from St. John's. This may indicate that the reduction in genetic variability, so far observed at neutral markers, has not affected invasion success. Previous work, which would seem to agree with this conclusion, have also shown that bottlenecks can reduce variability at neutral loci without effecting, or having only a small effect, on quantitative trait variation, the driving force behind adaptive variation (Dlugosch and Parker, 2008, Purcell *et al.*, 2012).

Contrary to what has been said in the previous paragraph, the case of the Salmonier River from the South Avalon showed a slightly different genetic makeup, in some analyses, from that seen for the other sample sites analysed. As supported by the ABC results, it is possible that an unrecorded, human mediated introduction event occurred at the site, possibly by transfer of eggs from the initial aggregations established in the St. John's region, or else these could be indicative of a surviving fish from a one-off introduction into the nearby Colinet River, which runs into the same bay as the Salmonier River. However, the Colinet River introduction was recorded as being composed of German trout ova, and none of the samples collected during the course of this study seem to be genetically related to the contemporary German samples analysed. It is also possible that the observed differences are as a result of stochastic processes and genetic drift acting on this introduced population.

Anadromous brown trout are more fecund than freshwater resident brown due to their much larger size at age (Poole *et al.*, 2006), so a new aggregation could hypothetically be founded by very low numbers of individuals. However, their low straying rate generally means new invasive populations would not be expected to be established by anadromous straying until a certain level of population density is reached in the donor catchment. This could be part of the reason why the brown trout's rate of spread in Newfoundland (approx. 4km/year) and other similar island habitats, i.e. the Kerguelan Islands (Ayllon *et al.*, 2006) is relatively slow, especially when compared with other studied invasions of salmonids e.g. chinook salmon in

South America: 54km/year, (Correa and Gross, 2008), chinook salmon in New Zealand: 13km/year (Kinnison *et al.,* 2008). Within their native range, it has been recorded that sea trout will recruit into vacant habitats, such as has been observed by the recolonisation of sea trout into the Tyne system in Britain (Milner *et al.,* 2004). Atlantic salmon have also, in recent times, in Britain and in other areas been recorded as beginning to recolonise restored habitats that were previously unsuitable due to factors such as high pollutant levels (Griffiths *et al.,* 2011, Ikediashi *et al.,* 2012). This suggests that straying in salmonid species is an effective method of colonising new habitats, both in existing and novel environments.

Although it was also reported that a small proportion of strains of brown trout sourced from German and English rivers were introduced to the St. John's region, at the same time that Scottish brown trout were being imported, our results appear to show no evidence of any other strain of brown trout successfully colonising the rivers studied here.

Brown trout have successfully spread on the island of Newfoundland over a period of 130 years, with the rate of spread not appearing to differ based on direction. Populations have been established up to 500km from the primary introduction site, although not all available habitats have been colonised by the invasive species (DFO, 2010), with the assumption being that the spread was mainly due to natural colonisation. A fully anadromous colonisation route combined with recorded short (<50km) marine migrations for brown trout populations in Newfoundland coupled with the fact that Newfoundland brown trout commonly exhibit skip-spawning (O'Connell, 1982), are all factors that could possibly slow expansion rates. However, our study showed that despite these limiting factors, viable, successful invasive populations have been established on the island of Newfoundland.

The existence of genetic structuring between rivers is similar to that observed in the species native range (Ferguson, 2006, Cauwelier *et al.,* 2011). The genetic differentiation observed here does appear to be quite high, with pairwise F_{ST} ranging from 0.09 in neighbouring invasive populations to 0.173 in more geographically distant samples. However, these are similar to levels of differentiation seen in other invasive populations of brown trout (Valiente *et al.,* 2007, pairwise F_{ST}= 0.072 to

0.175 in Patagonian rivers, Launey *et al.*, 2010, pairwise F_{ST}= 0 to 0.173 in rivers in the Kerguelen Islands).

Recorded History of Invasion

The study confirms that brown trout from the Howietoun hatchery in Scotland were the basis for the introduction of brown trout into Newfoundland at the end of the 19th century, as has been suggested by historical records (Maitland, 1887, Westley and Fleming, 2011). On the basis of two contemporary samples studied, the results suggest a continuing close relationship between the hatchery and Loch Leven itself over the course of the last century, as recorded in the hatchery records (Iain Semple, pers. comm.). Allelic composition in the two native Scottish samples is similar to that found in the various St. John's populations (Windsor Lake, Rennies and Virginia Rivers and the Waterford River) which suggests the source of the introductions of brown trout from the Howietoun hatchery to the St. John's area was mainly composed of Loch Leven fish.

Comparison with Ecological Investigations

Westley *et al.* (2012) examined the phenotypic divergence of the brown trout invasives in sixteen catchments across the Avalon Peninsula. The present study looked genetically at 12 of these catchments, finding strong evidence of genetic divergence and isolation by distance between sites following a pattern of dispersal from a centre point at St. John's. These results agree with, and provide more solid evidence for, the conclusions of this earlier work.

A conclusion of Westley and Fleming (2011), when examining the landscape factors affecting the brown trout invasion of Newfoundland, was that all catchments would be susceptible to invasion given enough time and high enough propagule pressure. However, with the current slow rate of spread and with reduced genetic diversity at increasing distance from the source of the introduction, it is suggested that Newfoundland trout would be considered unlikely to exhibit a large, sudden population explosion, as has been seen with other invasive species after a period of gradual invasion (Crooks and Soule, 2009). Due to their success in surviving and expanding since the initial introduction, it seems, however, reasonable to expect that brown trout will have a continued presence in Newfoundland.

Summary and Conclusions

Invasive brown trout in Newfoundland provide an unusually clear example of the genetic anatomy of a successful invasion process. The present study provides evidence that lowered genetic diversity has not yet impacted on the species' ability to colonise new habitats at increasing geographic distance for the original site of invasion. Brown trout are recognised as having a negative impact on native fish assemblages in areas where they have been introduced (O'Connell, 1982). This study adds to the knowledge on the expansion routes and rates of trout movement, an understanding of which is needed for successful management and control of this invasive, along with a clear pattern of the genetics of the invasion. It also validates the majority of the historical data collected on the invasion event in Newfoundland.

Our work also seems to support the theory presented by Westley and Fleming (2011) and Westley *et al.* (2012), based on their ecological investigations, in relation to the direction and means of invasion. The present study demonstrates how population molecular biology methods can complement ecological studies such as Westley *et al.* (2011) associated with the biology of invasive species.

We have presented evidence of the expansion pattern and genetic differences between established invasive populations of brown trout on Newfoundland, showing how populations can diverge from a common source over a short time period (130 years). The present study focused on differences between neutral genetic markers. It should be noted that these differences may not correlate with traits relating to fitness, such as reproductive success, survival, or phenotypic adaptations (controlled by adaptive loci). However, neutral markers have been utilised frequently to demonstrate differences between population groupings for management and conservation purposes (Beebee and Rowe, 2004, Allendorf and Luikart, 2007) and have an important role in addressing these issues.

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Appendix I, Chapter 3: Celtic Sea Trout Project Multiplex Panels

Panel 1:

PCR reaction volume	**3.5**	
Primer concentration pM/µl	**20**	
DNA volume	**1**	
Number of samples	**96**	
	x1	**x110**
PPP Mix	1.75	192.5
***Ssa85* forward**	0.007	0.77
***Ssa85* reverse**	0.007	0.77
***One102* forward**	0.014	1.54
***One102* reverse**	0.014	1.54
***Ssa406UOS* forward**	0.0385	4.235
***Ssa406UOS* reverse**	0.0385	4.235
***CAO54565* forward**	0.01225	1.3475
***CAO54565* reverse**	0.01225	1.3475
***CAO53293* forward**	0.0175	1.925
***CAO53293* reverse**	0.0175	1.925
***QUBStr2* forward**	0.02625	2.8875
***QUBStr2* reverse**	0.02625	2.8875
***One108* forward**	0.035	3.85
***One108* reverse**	0.035	3.85
***Ssa416* forward**	0.0105	1.155
***Ssa416* reverse**	0.0105	1.155
***One103* forward**	0.0175	1.925
***One103* reverse**	0.0175	1.925
***SsaD48* forward**	0.035	3.85
***SsaD48* reverse**	0.035	3.85
***Cocl-Lav-4* forward**	0.035	3.85
***Cocl-lav-4* forward**	0.035	3.85
***Oneu9ASC* forward**	0.00875	0.9625
***Oneu9ASC* reverse**	0.00875	0.9625
***CAO44828* forward**	0.014	1.54
***CAO44828* reverse**	0.014	1.54
H_20	0.2075	22.825

Panel 2:

PCR reaction volume	**3.5**	
Primer concentration pM/µl	**20**	
DNA volume	**1**	
Number of samples	**96**	
	x1	**x110**
PPP Mix	1.75	192.5
***Mhc1* forward**	0.00875	0.9625
***Mhc1* reverse**	0.00875	0.9625
***SsaD71* forward**	0.00875	0.9625
***SsaD71* reverse**	0.00875	0.9625
***SasaTAP2* forward**	0.014	1.54
***SasaTAP2* reverse**	0.014	1.54
***BG935488* forward**	0.007	0.77
***BG935488* reverse**	0.007	0.77
***Ssa410UOS* forward**	0.04375	4.8125
***Ssa410UOS* reverse**	0.04375	4.8125
***QUBStr3* forward**	0.0105	1.155
***QUBStr3* reverse**	0.0105	1.155
***CAO60177* forward**	0.035	3.85
***CAO60177* reverse**	0.035	3.85
***Ssa197* forward**	0.007	0.77
***Ssa197* reverse**	0.007	0.77
H_20	0.2075	22.825

Appendix II, Chapter 3: Summary statistics for population genetics parameters for sixteen loci.

Summary statistics for *S. trutta* samples screened for 16 microsatellite loci: ***N*** = number of individuals screened per sample: ***A*** (***T%***)= number of alleles (% observed in sample in relation to total observed among all samples), ***Ho*** = observed heterozygosity, ***He*** = expected heterozygosity (Nei, 1987), ***Ar*** = Allelic richness following the rarefaction method (Petit *et al.*, 1998), **HWE** = Significance of exact tests for non-conformance to Hardy-Weinberg Expectations (Guo & Thompson, 1992), ns=not significant. Significant values after Bonferroni correction (0.05/16=0.003) are denoted by *, with highly significant values (p<0.001 equivalent) denoted by **. Average values per locus are also provided.

Region	Site		Ssa416	Cocl-Lav-4	One9uASC	CA048828	Ssa85	One102-a	One102-b	Ssa406UOS	CA054565	Str2QUB	Str3QUB	CA060177	Ssa197	SsaD71	SaSaTAP2A	Ssa410UOS	Avg.	Total NA
Scotland	Lough Leven Wild	N	34	37	37	38	38	38	38	38	38	38	38	38	36	36	38	37	37.3	
		A	3	6	6	19	4	2	13	23	2	17	5	10	7	11	6	26	10.0	160
		At%	100	60	54.5	54.3	33.3	100	52	47.9	33.3	48.6	62.5	52.6	36.8	61.1	54.5	38.8	48.6	
		A_R	3.0	6.0	5.9	18.6	4.0	2.0	12.9	22.1	1.9	16.5	4.9	9.9	6.9	11.0	6.0	25.1	-	
		H_O	0.324	0.730	0.595	0.947	0.790	0.342	0.947	0.895	0.026	0.790	0.579	0.790	0.694	0.889	0.658	0.919	0.7	
		H_E	0.319	0.758	0.725	0.919	0.689	0.380	0.894	0.926	0.026	0.887	0.588	0.832	0.729	0.839	0.653	0.950	0.7	
		HWE	ns	ns	ns	ns	ns	ns	ns	ns	-	ns	ns	ns	ns	ns	ns	ns		
Scotland	Howietoun 2011 Hatchery	N	68	66	64	67	68	65	68	57	68	63	68	64	68	68	68	68	66.1	
		A	3	6	5	16	4	2	15	21	3	17	5	9	9	10	7	24	9.8	156
		At%	100	60	45.5	45.7	33.3	100	60	43.8	50	48.6	62.5	47.4	47.4	55.6	63.6	35.8	47.4	
		A_R	2.7	5.5	4.5	13.7	3.5	2.0	13.4	18.1	3.0	15.2	4.5	7.7	6.9	9.5	7.0	18.9	-	
		H_O	0.132	0.727	0.641	0.791	0.529	0.569	0.912	0.754	0.294	0.889	0.603	0.688	0.779	0.750	0.691	0.882	0.665	
		H_E	0.126	0.770	0.726	0.898	0.558	0.481	0.900	0.913	0.367	0.907	0.591	0.651	0.745	0.822	0.690	0.925	0.692	
		HWE	ns	ns	ns	ns	ns	ns	ns	**	ns	ns	ns	ns	ns	ns	ns	**		
St. John's	Windsor Parker's Brook	N	85	71	90	90	90	89	90	89	86	90	89	89	83	87	90	90	87.4	
		A	2	5	5	14	5	2	16	16	3	10	3	6	6	12	5	24	8.4	134
		At%	66.7	50	45.5	40	41.7	100	64	33.3	50	28.6	37.5	31.6	31.6	66.7	45.5	35.8	40.7	
		A_R	1.9	5.0	4.4	12.7	4.4	2.0	14.7	13.0	2.9	9.7	3.0	5.4	4.8	10.0	5.0	18.4	-	
		H_O	0.047	0.521	0.689	0.889	0.733	0.157	0.989	0.944	0.012	0.889	0.539	0.843	0.675	0.874	0.700	0.978	0.655	
		H_E	0.046	0.710	0.721	0.892	0.743	0.146	0.915	0.887	0.164	0.816	0.536	0.772	0.684	0.853	0.674	0.916	0.655	
		HWE	ns	*	ns	**	ns	ns	**	*	**	ns	ns	ns	ns	ns	ns	**		

Region	Site		Ssa416	Cocl-Lav-4	One9uASC	CA048828	Ssa85	One102-a	One102-b	Ssa406UOS	CA054565	Str2QUB	Str3QUB	CA060177	Ssa197	SsaD71	SaSaTAP2A	Ssa410UOS	Avg.	Total	NA
St. John's	Windsor Middle Rocky Brook	N	93	94	95	95	95	95	95	95	95	95	95	93	94	94	94	93	94.4		
		A	2	5	5	14	4	2	15	20	1	10	3	6	4	11	6	23	8.2	131	
		At%	66.7	50	45.5	40	33.3	100	60	41.7	16.7	28.6	37.5	31.6	21.1	61.1	54.5	34.3	39.8		
		A_R	1.7	5.0	5.0	13.0	4.0	2.0	13.8	16.8	1.0	8.7	3.0	5.4	4.0	8.9	5.4	18.3	-		
		H_O	0.032	0.511	0.695	0.884	0.716	0.168	0.916	0.916	0.000	0.811	0.453	0.774	0.745	0.872	0.713	0.957	0.635		
		H_E	0.032	0.747	0.698	0.888	0.703	0.172	0.912	0.915	0.000	0.832	0.461	0.750	0.676	0.844	0.723	0.924	0.642		
		HWE	ns	**	ns	ns	ns	ns	ns	ns	-	ns	ns	ns	ns	ns	ns	**			
St. John's	Rennie River	N	96	96	95	96	96	95	94	96	95	96	96	94	95	96	96	96	95.5		
		A	3	8	9	22	6	2	15	28	4	20	6	10	6	13	6	27	11.6	185	
		At%	100	80	81.8	62.9	50	100	60	58.3	66.7	57.1	75	52.6	31.6	72.2	54.5	40.3	56.2		
		A_R	3	7.2	7.8	16.8	4.6	2	12.4	21.2	3.5	16.4	4.9	8.5	5.5	11.9	5.9	20.5	-		
		H_O	0.281	0.635	0.779	0.938	0.552	0.232	0.851	0.854	0.074	0.906	0.563	0.692	0.674	0.885	0.76	0.948	0.664		
		H_E	0.268	0.731	0.782	0.901	0.592	0.206	0.875	0.897	0.15	0.918	0.594	0.77	0.669	0.863	0.754	0.933	0.681		
		HWE	ns	ns	ns	ns	ns	ns	ns	*	**	ns	ns	**	ns	ns	ns	ns			
St. John's	Virginia River	N	86	86	86	86	86	86	86	85	86	83	86	81	86	76	73	86	84		
		A	3	6	7	19	3	2	17	24	2	17	5	8	6	12	7	27	10.3	165	
		At%	100	60	63.6	54.3	25	100	68	50	33.3	48.6	62.5	42.1	31.6	66.7	63.6	40.3	50.2		
		A_R	3	6	6.8	16	3	2	13.6	18.1	1.6	14.4	5	7.7	5	10.5	6.8	19.4	-		
		H_O	0.349	0.802	0.837	0.93	0.628	0.326	0.861	0.929	0.023	0.88	0.628	0.79	0.767	0.671	0.658	0.872	0.684		
		H_E	0.348	0.776	0.752	0.905	0.628	0.305	0.84	0.898	0.023	0.895	0.62	0.764	0.718	0.739	0.776	0.91	0.681		
		HWE	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns			
St. John's	Waterford River	N	95	95	96	96	95	92	96	96	96	96	96	92	96	89	96	96	94.88		
		A	3	5	6	17	4	2	11	20	2	8	4	7	7	11	6	25	8.6	138	
		At%	100	50	54.5	48.6	33.3	100	44	41.7	33.3	22.9	50	36.8	36.8	61.1	54.5	37.3	41.9		
		A_R	2.9	5	4.9	14.6	3.7	2	9.6	16.7	2	7.8	4	6	6.1	10.6	5.5	18.9	-		
		H_O	0.126	0.758	0.719	0.875	0.4	0.196	0.854	0.906	0	0.792	0.573	0.533	0.802	0.82	0.563	0.875	0.612		
		H_E	0.121	0.766	0.729	0.892	0.489	0.244	0.837	0.924	0.08	0.818	0.608	0.524	0.764	0.856	0.572	0.914	0.634		
		HWE	ns	ns	ns	ns	ns	ns	ns	ns	**	ns	ns	ns	ns	ns	ns	**			
North	Salmon Cove	N	99	97	99	99	99	99	99	99	99	99	99	97	98	97	99	98	98.5		
		A	3	6	6	11	3	2	16	16	1	13	4	7	4	12	6	28	8.6	138	
		At%	100	60	54.5	31.4	25	100	64	33.3	16.7	37.1	50	36.8	21.1	66.7	54.5	41.8	41.9		
		A_R	3	5.3	5.4	9.1	3	2	13.1	13.1	1	10.3	3.9	6.1	4	11	5.4	20.3	-		
		H_O	0.354	0.711	0.566	0.828	0.647	0.293	0.859	0.818	0	0.566	0.424	0.629	0.694	0.907	0.535	0.949	0.611		
		H_F	0.365	0.742	0.649	0.822	0.649	0.279	0.888	0.864	0	0.601	0.436	0.657	0.704	0.877	0.6	0.915	0.628		
		HWE	ns	ns	ns	ns	ns	ns	ns	ns	-	ns	ns	ns	ns	ns	ns	**			

Region	Site		Ssa416	Cocl-Lav-4	One9uASC	CA048828	Ssa85	One102-a	One102-b	Ssa406UOS	CA054565	Str2QUB	Str3QUB	CA060177	Ssa197	SsaD71	SaSaTAP2A	Ssa410UOS	Avg.	Total	NA
North	Chapel Arm	N	98	91	97	98	98	97	97	97	98	98	98	96	96	98	98	98	97.1		
		A	2	6	6	6	4	2	12	9	4	8	5	8	4	6	4	16	6.4	102	
		At%	66.7	60	54.5	17.1	33.3	100	48	18.8	66.7	22.9	62.5	42.1	21.1	33.3	36.4	23.9	31		
		A_R	2	4.7	4.7	4.7	3.6	2	10.7	6.7	2.7	7.4	3.7	5.9	4	6	2.9	11.2	-		
		H_O	0.194	0.473	0.701	0.765	0.602	0.124	0.794	0.68	0.122	0.745	0.571	0.656	0.573	0.714	0.255	0.776	0.547		
		H_F	0.208	0.505	0.695	0.723	0.646	0.117	0.85	0.698	0.117	0.718	0.559	0.708	0.635	0.802	0.25	0.802	0.565		
		HWE	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns			
North	Port Rexton	N	88	86	84	87	88	84	88	88	88	88	87	85	82	85	87	88	86.44		
		A	1	4	4	7	3	1	12	11	2	8	2	6	4	6	5	17	5.8	93	
		At%	33.3	40	36.4	20	25	50	48	22.9	33.3	22.9	25	31.6	21.1	33.3	45.5	25.4	28.3		
		A_R	1	4	3.6	6.7	3	1	8.9	8.9	2	7.8	2	5.2	3.5	5.8	4.5	13.8	-		
		H_O	0	0.663	0.56	0.644	0.466	0	0.761	0.784	0.216	0.739	0.494	0.682	0.488	0.718	0.287	0.932	0.527		
		H_F	0	0.723	0.534	0.68	0.489	0	0.798	0.749	0.229	0.81	0.451	0.696	0.517	0.675	0.305	0.844	0.531		
		HWE	-	ns	ns	ns	ns	-	ns	ns	ns	ns	ns	*	ns	ns	ns	**			
South	Chance Cove	N	97	98	95	98	98	98	98	97	98	98	98	96	98	92	98	98	97.2		
		A	1	8	7	11	7	2	11	14	4	11	4	7	4	10	4	18	7.7	123	
		At%	33.3	80	63.6	31.4	58.3	100	44	29.2	66.7	31.4	50	36.8	21.1	55.6	36.4	26.9	37.4		
		A_R	1	7.2	6.1	8.5	5.2	2	8.3	10	3	9.1	3.3	5.7	3.9	9.3	2.7	13.5	-		
		H_O	0	0.674	0.642	0.837	0.663	0.122	0.684	0.742	0.102	0.776	0.469	0.552	0.694	0.837	0.327	0.816	0.559		
		H_F	0	0.769	0.69	0.784	0.641	0.116	0.731	0.811	0.099	0.825	0.442	0.586	0.684	0.87	0.343	0.832	0.576		
		HWE	-	ns	**	ns	ns	ns	ns	ns	ns	**	ns	ns	ns	ns	ns	**			
South	Salmonier No Name Brook	N	91	93	93	93	93	93	93	93	91	93	93	89	85	87	92	92	91.5		
		A	1	6	6	10	5	2	10	12	3	10	5	7	6	5	4	13	6.6	105	
		At%	33.3	60	54.5	28.6	41.7	100	40	25	50	28.6	62.5	36.8	31.6	27.8	36.4	19.4	31.9		
		A_R	1	5.1	5.3	9.4	4.1	2	9.1	10.8	3	9.4	5	9.4	6.4	8.2	4.2	13.3	-		
		H_O	0	0.634	0.688	0.871	0.419	0	0.72	1	0.088	0.893	0.677	0.742	0.624	0.701	0.446	0.837	0.588		
		H_F	0	0.637	0.725	0.798	0.585	0.352	0.741	0.859	0.126	0.836	0.65	0.772	0.698	0.758	0.492	0.826	0.616		
		HWE	-	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	**	ns	ns	ns	ns			
South	South East Placentia	N	95	93	95	95	95	92	94	95	94	95	95	93	86	93	95	94	93.7		
		A	1	6	5	6	4	2	9	8	2	8	4	6	5	5	4	8	5.2	83	
		At%	33.3	60	45.5	17.1	33.3	100	36	16.7	33.3	22.9	50	31.6	26.3	27.8	36.4	11.9	25.2		
		A_R	1	6.5	4.2	5.6	3.7	1.6	7.8	7.5	2.3	7.8	3.6	6.1	5.4	4.9	4	6.6	-		
		H_O	0	0.677	0.505	0.684	0.695	0.022	0.819	0.768	0.021	0.842	0.558	0.559	0.744	0.613	0.526	0.649	0.543		
		H_F	0	0.672	0.656	0.741	0.666	0.022	0.824	0.783	0.042	0.799	0.594	0.622	0.765	0.621	0.562	0.715	0.568		
		HWE	-	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**	ns	ns	ns	ns			

Appendix III, Chapter 3: Allele frequencies and allelic compositions of all loci at all sites in both Scotland and Newfoundland.

Populations	*Ssa416* Alleles			Genes
	122	131	140	
Scotland				
Loch Leven	1.50	17.60	80.90	68
Howietoun	5.10	1.50	93.40	136
St. John's				
Windsor-Parkers Br.		2.40	97.60	170
Windsor-Middle Rocky Br.		1.60	98.40	186
Rennies River	10.40	4.70	84.90	192
Virginia River	16.30	4.70	79.10	172
Waterford River	3.20	3.20	93.70	190
North Avalon				
Salmon Cove	11.10	10.60	78.30	198
Chapel Arm		11.70	88.30	196
Port Rexton			100.00	176
South Avalon				
Chance Cove			100.00	194
Salmonier			100.00	180
S E Placentia River			100.00	190

Populations	Alleles	Locus: Cocl-Lav-4						Genes
	147	153	155	157	159	161	163	
Scotland								
Loch Leven		17.60	18.90	37.80	20.30	2.70	2.70	74
Howietoun		6.10	21.20	31.80	25.00	15.20		132
St. John's								
Windsor-Parkers Br.		4.90	40.10	30.30	19.00	5.60		142
Windsor-Middle Rocky Br.		13.80	34.60	16.50	29.80	5.30		188
Rennies River	1.00	13.50	16.10	45.80	7.30	9.90	5.20	192
Virginia River		5.20	23.80	33.70	19.20	5.80	12.20	172
Waterford River		16.30	15.80	29.50	7.90	30.50		190
North Avalon								
Salmon Cove		38.70	22.20	22.20	9.30	7.20	0.50	194
Chapel Arm	0.50	68.10	7.70	8.80		14.30		182
Port Rexton		30.80	34.30	23.80	11.00			172
South Avalon								
Chance Cove	4.60	6.10	8.20	32.10	31.10	2.60	14.80	196
Salmonier	0.50	9.80	53.30	26.60	8.20		1.60	184
S E Placentia River	1.10	6.50	7.00	52.70	15.60	3.20	14.00	186

Populations	Alleles	One9uASC								Genes
	188	192	194	200	202	204	206	208	210	
Scotland										
Loch Leven				13.50	23.00	43.20	16.20	1.40	2.70	74
Howietoun				18.80	21.10	40.60	18.80		0.80	128
St. John's										
Windsor-Parkers Br.				11.70	33.30	34.40	20.00	0.60		180
Windsor-Middle Rocky Br.	3.70			11.10	42.10	32.10	11.10			190
Rennies River	12.60	1.10		9.50	16.80	38.90	10.50	7.40	2.60	190
Virginia River	5.80			7.60	25.00	16.30	39.00	2.30	4.10	172
Waterford River	1.00			38.50	20.30	25.50	14.10	0.50		192
North Avalon										
Salmon Cove				2.00	5.60	45.50	36.90		9.10	198
Chapel Arm		0.50	0.50		19.10	21.10	45.90		12.90	194
Port Rexton				1.20	6.00	59.50	33.30			168
South Avalon										
Chance Cove		0.50	3.70	2.60	17.90	45.80	3.20		26.30	190
Salmonier			0.50	28.80	5.40	34.20	28.30		2.70	184
S E Placentia River		1.10			25.80	46.30	0.00		25.80	190

Populations	CA048828 Alleles										
	250	252	256	258	262	264	266	268	270	272	274
Scotland											
Loch Leven				9.20	13.20	2.60	5.30	3.90	3.90	2.60	19.70
Howietoun			12.70	3.70	3.70	11.20	3.00	0.70	0.70	18.70	13.40
St. John's											
Windsor-Parkers Br.				11.70	7.80		2.80	20.00	10.60	1.70	15.60
Windsor-Middle Rocky Br.				5.80	6.80		5.30	24.70	10.00	1.60	6.80
Rennies River	0.50	0.50		7.80	7.80		20.80	0.50		0.50	2.60
Virginia River				10.50	10.50		12.20	3.50	3.50	4.70	3.50
Waterford River				8.30	3.60		14.10	7.80	2.10	3.60	2.10
North Avalon											
Salmon Cove					20.20	0.50	15.70	0.50		4.00	8.60
Chapel Arm		0.50	0.50				40.30				24.50
Port Rexton					3.40		12.60		14.40		
South Avalon											
Chance Cove	1.50	0.50	2.60	0.50	4.60		34.20	0.50			
Salmonier	0.50			9.80	7.10		6.50	3.30	1.60		1.10
S E Placentia River	0.50	0.50			14.20		31.10	2.60			

Populations	Alleles											Genes
	276	278	280	282	283	286	287	288	289	292	298	
Scotland												
Loch Leven	2.60	5.30	2.60	3.90			10.50	2.60		5.30		76
Howietoun	10.40	4.50	1.50	6.70		0.70	7.50	0.70				134
St. John's												
Windsor-Parkers Br.	3.90	4.40	1.70		5.60		7.20	0.00	0.60	6.70		180
Windsor-Middle Rocky Br.	5.80	3.20	5.80		4.70		12.10	0.00	0.50	6.80		190
Rennies River	2.10	15.60	1.60	4.20	7.80		3.60	3.10	1.60	0.00	1.60	192
Virginia River	1.20	19.20	2.90	5.80		1.20	11.60	1.20	0.60	1.70		172
Waterford River		1.00	7.30		1.60	7.80	23.40	2.10	5.70	0.00	7.80	192
North Avalon												
Salmon Cove	1.50	28.30					16.20	2.50		2.00		198
Chapel Arm		17.90					16.30					196
Port Rexton	1.70	52.30	6.30				9.20					174
South Avalon												
Chance Cove	11.70	19.90	2.60				21.40					196
Salmonier	14.70	17.40					37.00			0.50		184
S E Placentia River	0.00	34.20					16.80					190

Populations	Locus: One102-a Alleles 167	170	Genes
Scotland			
Lough Leven	25.00	75.00	76
Howietoun	39.20	60.80	130
St. John's			
Windsor-Parkers Br.	7.90	92.10	178
Windsor-Middle Rocky Br.	9.50	90.50	190
Rennies River	11.60	88.40	190
Virginia River	18.60	81.40	172
Waterford River	14.10	85.90	184
North Avalon			
Salmon Cove	16.70	83.30	198
Chapel Arm	6.20	93.80	194
Port Rexton		100.00	168
South Avalon			
Chance Cove	6.10	93.90	196
Salmonier	22.80	77.20	184
S E Placentia River	1.10	98.90	184

Populations	One102-b Alleles 191	195	199	203	207	211	215	219	223	227	237
Scotland											
Loch Leven		18.40		7.90	6.60	3.90	21.10	7.90	2.60	5.30	7.9
Howietoun		11.00	3.70	18.40	5.90	12.50	8.10	14.00	4.40	1.50	5.9
St. John's											
Windsor-Parkers Br.		5.60	1.10	14.40	7.20	6.10	6.70	10.00	5.60	2.80	
Windsor-Middle Rocky Br.		15.30	1.60	5.30	9.50	4.20	11.10	11.10	0.00	4.70	
Rennies River		6.90	5.90	7.40	12.20	23.40	14.40	14.40	1.60	1.10	0.5
Virginia River	0.60	7.00	1.20	4.10	14.50	7.00	20.30	29.70	2.90	1.20	
Waterford River		18.80		2.10	7.30	0.50	25.50	1.00	0.00	19.30	
North Avalon											
Salmon Cove	12.60	11.10		4.00		3.00	16.70	4.50		1.50	0.5
Chapel Arm	7.70	25.80		2.10		4.60	1.00	8.20	1.00	19.10	16.5
Port Rexton	13.10	10.80		0.60		5.70	7.40	31.20		26.70	1.7
South Avalon											
Chance Cove		3.60			5.10	43.90		0.50		0.50	
Salmonier		6.00				3.80	3.30	4.30			
S E Placentia River					22.90	25.00		2.10		16.00	

Populations	Alleles									Genes
	239	241	243	247	251	255	259	267	283	
Scotland										
Loch Leven		3.90	9.20	1.30	3.90					76
Howietoun		3.70		8.10	1.50	0.70	0.70			136
St. John's										
Windsor-Parkers Br.		4.40	15.60	9.40	2.80	2.80	0.60	5.00		180
Windsor-Middle Rocky Br.	4.70	0.50	6.80	11.60	3.20	8.40		2.10		190
Rennies River		1.10	4.30	4.80		1.10			1.10	188
Virginia River	1.20		2.30	1.70	1.70	0.60			2.90	172
Waterford River		5.70		3.60			13.50		2.60	192
North Avalon										
Salmon Cove		4.50	19.70	8.10	3.00	8.60	0.50		1.00	198
Chapel Arm			8.20			3.60	2.10			194
Port Rexton					1.10	0.60			0.60	176
South Avalon										
Chance Cove		0.50	6.60	23.00		13.80				196
Salmonier	1.10		2.70	39.10	7.60	31.00				184
S E Placentia River			16.00	9.60	6.90	1.10				188

Population	Ssa406UOS Alleles										
	433	437	439	441	443	445	447	449	451	455	457
Scotland											
Loch Leven		10.50	15.80		2.60				3.90	3.90	2.60
Howietoun		0.90	9.60		14.00		3.50	0.90	2.60	13.20	
St. John's											
Windsor-Parkers Br.	4.50	0.60	2.20						15.20	17.40	
Windsor-Middle Rocky Br.	6.30	2.60	4.20			0.50			14.20	10.00	
Rennies River	1.00	4.20	1.60		3.10	0.50	1.60	2.10	6.80	3.60	2.10
Virginia River	0.60	3.50	2.90	1.20	0.60	2.40		0.60	19.40	2.90	
Waterford River	7.80	6.80	8.30	5.70	4.70		0.50	16.70		9.90	
North Avalon											
Salmon Cove	8.10	4.50			4.50	5.60			3.00	16.20	2.00
Chapel Arm					0.50						
Port Rexton		1.70				0.60				0.60	
South Avalon											
Chance Cove	1.00					0.50			0.50	21.10	
Salmonier	8.70	2.20				1.60	0.50		6.50	19.00	
S E Placentia River						4.70				10.00	

Population	Alleles										
	459	463	467	469	471	475	479	483	487	489	491
Scotland											
Loch Leven	17.10	5.30	2.60		6.60	1.30	3.90	1.30	2.60		1.3
Howietoun	3.50	6.10			4.40	0.90		1.80			2.6
St. John's											
Windsor-Parkers Br.	12.90	9.00	0.60		16.30	6.70				1.10	1.1
Windsor-Middle Rocky Br.	17.40	9.50	2.60		5.80	3.70	2.10			2.10	5.8
Rennies River	13.50	26.00	5.70	3.60	3.10	1.00	2.60		1.00	0.50	2.1
Virginia River	17.60	7.10	14.10	1.80	1.80		3.50		5.90	2.40	0.6
Waterford River	2.60	7.80	2.60	0.50	5.20	2.10	7.30		3.10	0.00	2.1
North Avalon											
Salmon Cove	13.60	3.00			0.50	1.00					
Chapel Arm	45.90					4.60	0.50				
Port Rexton	13.60	5.70			1.10	0.00					
South Avalon											
Chance Cove	24.20				0.50	1.50					25.8
Salmonier	8.70				2.70						19.6
S E Placentia River	35.30				0.00				1.10		17.9

Ssa406UOS continued

Population	Alleles									Genes
	495	499	501	503	505	509	511	513	515	
Scotland										
Loch Leven		5.30		1.30	1.30		1.30	3.90		76
Howietoun	17.50	1.80	4.40	1.80	0.00		1.80			114
St. John's										
Windsor-Parkers Br.				6.20	3.90					178
Windsor-Middle Rocky Br.		2.10	0.50	5.30	3.70					190
Rennies River	1.00	2.60		5.20	2.10			1.00		192
Virginia River	0.60	4.10		4.10					1.20	170
Waterford River			0.50		0.50					192
North Avalon										
Salmon Cove			6.60		27.30	0.50		3.00		198
Chapel Arm					18.60	4.10	1.50	23.70		194
Port Rexton			2.80		36.90		4.00	2.30	30.70	176
South Avalon										
Chance Cove	0.50		1.50		13.90				4.60	194
Salmonier	8.20				20.70			1.10		184
S E Placentia River				1.10	21.60			0.50	7.90	190

Locus: CA054565

Populations	Alleles				Genes
	113	115	117	119	
Scotland					
Loch Leven	98.70		1.30		76
Howietoun	77.90	6.60	15.40		136
St. John's					
Windsor-Parkers Br.	91.30	5.20			172
Windsor-Middle Rocky Br.	100.00				190
Rennies River	92.10	4.20	2.60		190
Virginia River	98.80		1.20		172
Waterford River	95.80	4.20			192
North Avalon					
Salmon Cove	100.00				198
Chapel Arm	93.90		5.10	0.50	196
Port Rexton	86.90		13.10		176
South Avalon					
Chance Cove	94.90		0.50	4.60	196
Salmonier	94.40	1.10	3.90	0.60	180
S E Placentia River	97.90	1.10	0.00	1.00	188

Population	Alleles	Str2QUB									
	213	219	223	225	227	231	235	243	245	257	271
Scotland											
Loch Leven	18.40	3.90	2.60	1.30	2.60		1.30				18.40
Howietoun	5.60	4.80	6.30	19.00	8.70		2.40	0.80			7.90
St. John's											
Windsor-Parkers Br.	5.00	30.00	5.60					7.20			25.00
Windsor-Middle Rocky Br.	16.30	28.40	1.10					6.80			16.30
Rennies River	10.40	1.00		4.20	1.60	9.90	1.60	0.00	2.60	3.60	16.70
Virginia River	14.50	1.80		1.80	4.20	3.00			1.20	1.20	15.10
Waterford River	28.10	14.10		2.10							25.00
North Avalon											
Salmon Cove	61.10	4.00		1.00			1.00				2.00
Chapel Arm	46.90	1.50						2.60			16.30
Port Rexton	29.50	4.50						18.80			2.80
South Avalon											
Chance Cove	3.10	1.50						4.10			10.70
Salmonier	25.50	23.90						0.50			10.30
S E Placentia River								22.10			

Population	Alleles									Genes
	279	287	297	299	303	309	311	313	315	323	
Scotland											
Loch Leven	5.30	7.90	19.70		2.60	1.30		5.30	1.30	2.60	76
Howietoun	0.00	0.00	2.40	15.90		0.80		6.30			126
St. John's											
Windsor-Parkers Br.	14.40		3.90	3.90				2.20			180
Windsor-Middle Rocky Br.	13.20		2.60	12.10			0.50	2.60			190
Rennies River	6.80	9.40	6.80	4.70			9.90	4.20	0.50	0.50	192
Virginia River	12.70	6.60	4.20	9.60	0.60		2.40	17.50			166
Waterford River	8.30	0.00		8.90				5.70		7.80	192
North Avalon											
Salmon Cove	4.00	1.50	14.60	3.50	2.50			3.00	0.50		198
Chapel Arm	5.60		2.00	8.70	16.30						196
Port Rexton	0.00	6.80	21.60	0.00							176
South Avalon											
Chance Cove	12.80	1.00	16.80	1.00	0.50			20.90	27.60		196
Salmonier	14.70	1.60	10.30	3.80	1.10			6.50	1.60		184
S E Placentia River	3.20	5.80	6.80	6.80				24.70	28.40		190

		Str3QUB						
Population	Alleles							Genes
	125	129	141	145	157	169	181	
Scotland								
Loch Leven		17.10		2.60	59.20	19.70		76
Howietoun		9.60		0.70	58.10	25.00	6.60	136
St. John's								
Windsor-Parkers Br.		28.70			61.20	10.10		178
Windsor-Middle Rocky Br.		13.20			70.50	16.30		190
Rennies River		6.20	1.00	0.50	58.90	20.80	12.50	192
Virginia River		4.70			54.10	28.50	5.80	172
Waterford River		8.90			57.30	14.10	19.80	192
North Avalon								
Salmon Cove		11.60			73.20	12.60	2.50	198
Chapel Arm	0.50	48.50	0.50		45.40	5.10		196
Port Rexton		33.90			66.10			174
South Avalon								
Chance Cove		25.00	0.50		70.40	4.10		196
Salmonier	2.20	37.00	1.60		44.00	14.70		184
S E Placentia River		52.60	1.10		34.20	12.10		190

Population	Alleles					CA060177						Genes
		251	259	263	267	271	275	279	283	287	291	299	
Scotland												
Loch Leven		2.60	14.50	7.90	31.60	17.10	13.20	3.90	3.90	3.90			76
Howietoun		1.60	53.10		23.40	1.60	4.70	11.70	1.60		1.60		128
St. John's													
Windsor-Parkers Br.			30.90	12.90	28.70	16.30			10.70	0.60			178
Windsor-Middle Rocky Br.			24.20	15.10	37.60	16.70	0.50		5.90				186
Rennies River			6.40	26.60	35.10	17.60	2.10	5.90	2.70	0.50	1.10		188
Virginia River			11.70	19.80	40.70	11.70	3.10	6.80	4.30	1.90			162
Waterford River			7.60	6.50	67.40	0.50	8.70		1.10	8.20			184
North Avalon													
Salmon Cove			16.00	17.50	53.10	8.80	2.10		1.00	1.50			194
Chapel Arm			27.60	0.50	40.10	23.40	0.50		5.70			1.00	192
Port Rexton			10.60	0.60	1.80	45.90	18.20		22.90				170
South Avalon													
Chance Cove			40.60		50.00	2.10	3.60	1.60		1.00			192
Salmonier			11.90		38.60	23.90	8.00	0.60	1.10	8.50		2.30	176
S E Placentia River			5.40		52.70	5.40	31.20			2.70		0.50	186

Population	Alleles				Ssa197				Genes
		130	134	138	142	146	154	174	
Scotland									
Loch Leven		5.60	38.90	23.60	26.40	2.80	1.40		72
Howietoun		14.00	36.00	30.10	14.00			2.90	136
St. John's									
Windsor-Parkers Br.		12.70	47.00	13.90	25.30				166
Windsor-Middle Rocky Br.		18.60	47.30	9.60	24.50				188
Rennies River		6.80	47.40	30.50	10.50		3.70		190
Virginia River		7.00	27.30	35.50	28.50	1.20		0.60	172
Waterford River		13.00	20.80	25.50	33.30	1.60	5.20		192
North Avalon									
Salmon Cove		10.20	18.40	35.20	36.20				196
Chapel Arm		5.70	33.30	49.00	12.00				192
Port Rexton		1.20	37.80	58.50	2.40				164
South Avalon									
Chance Cove		30.10	36.70	2.60	30.60				196
Salmonier		10.70	16.10	47.60	19.00		4.20	1.20	168
S E Placentia River		23.30	27.90	8.70	9.30		30.20		172

Population	Alleles	Genes	SsaD71													Genes
	185	189	193	197	201	205	209	213	217	221	225	229	233	237	241	
Scotland																
Loch Leven	34.70	8.30	12.50	6.90			4.20	9.70	8.30			5.60	2.80	2.80	4.20	72
Howietoun	22.10	11.80	2.20	30.90		3.70	0.00	8.10	11.80	1.50	5.10		2.90			136
St. John's																
Windsor-Parkers Br.	12.60	1.10	22.40	17.20	0.60		17.80	13.20	4.60		5.20	0.60		1.10	3.40	174
Windsor-Middle Rocky Br.	22.30	3.70	20.20	13.80	0.50		16.50	12.80	6.90		0.50	0.50			2.10	188
Rennies River	16.70	5.70	3.10	7.30	2.10	6.80	2.10	3.60	27.10	8.90	12.00	0.50			4.20	192
Virginia River	46.10	5.90	17.10	1.30	2.60	2.60	2.60	4.60	12.50	2.00		0.70			2.00	152
Waterford River	12.40	28.10	7.90	8.40	3.40			5.60	16.30		6.20	2.20		2.20	7.30	178
North Avalon																
Salmon Cove	5.20	6.70	2.10	14.40		2.60	1.50	19.60	10.30	16.00	1.00	13.40			7.20	194
Chapel Arm		5.10	19.90	14.80				30.60				16.80			12.8	196
Port Rexton		19.40	51.20	4.10				12.40	1.80						11.2	170
South Avalon																
Chance Cove	17.40	0.00	15.20	12.50				3.80		3.30		14.10		4.30	15.2	184
Salmonier	1.20	6.40	14.50	31.40		1.20		1.70	7.60			1.70			33.7	172
S E Placentia River		0.00	14.00	19.40				7.50				2.70			56.5	186

Population	Alleles	SaSaTAP2A						Genes
	287	316	320	324	326	328	330	
Scotland								
Loch Leven	9.20	17.10		3.90	55.30	9.20	5.30	76
Howietoun		14.70	6.60	5.90	51.50	13.20	4.40	136
St. John's								
Windsor-Parkers Br.	12.20	17.20		8.30	51.70	10.60		180
Windsor-Middle Rocky Br.	9.00	17.00		13.80	45.20	14.40		188
Rennies River	15.60	15.60		3.10	41.10	9.90	14.60	192
Virginia River	19.90	7.50	2.10	30.10	29.50	4.10	6.80	146
Waterford River	1.00	11.50		6.80	62.50	14.60	3.60	192
North Avalon								
Salmon Cove	7.60	28.30	2.00	1.00	56.10	5.10		198
Chapel Arm	0.00	12.80		1.00	85.70			196
Port Rexton	4.00	9.80	1.10	2.30	82.80			174
South Avalon								
Chance Cove		20.40	0.50	0.50	78.60			196
Salmonier		6.00	2.20		66.50	24.70		182
S E Placentia River	3.70	11.10			60.50	24.70		190

Locus:					Ssa410UOS					
Population	**Alleles**									
	182	**186**	**190**	**202**	**206**	**210**	**216**	**220**	**224**	**226**
Scotland										
Loch Leven			2.70	9.50	1.40			13.50	5.40	2.70
Howietoun				2.90	0.70		11.00	13.20	1.50	0.00
St. John's										
Windsor-Parkers Br.	2.20				0.60	3.30	13.90	3.30	18.90	
Windsor-Middle Rocky Br.	3.80		0.50		2.20	2.20	9.70	8.10	16.10	
Rennies River		1.00	4.20		4.70	0.50	6.80	5.70	0.50	3.10
Virginia River	1.70	7.60	1.20		0.00	1.70	17.40	0.60	2.30	2.90
Waterford River	6.20				2.60	5.20	6.20	1.00	3.10	2.10
North Avalon										
Salmon Cove							16.80	0.50	16.80	
Chapel Arm						0.50	35.70		16.30	
Port Rexton							11.40		34.10	
South Avalon										
Chance Cove				25.00			1.00		4.60	
Salmonier		1.10		9.90					1.10	
S E Placentia River				40.40			6.90		0.00	

Population	**Alleles**											
	232	**236**	**240**	**245**	**249**	**253**	**257**	**262**	**264**	**266**	**270**	**274**
Scotland												
Loch Leven	1.40	1.40	0.00	5.40	1.40				1.40		5.4	6.8
Howietoun	5.10	2.90	0.70	12.50	0.70	1.50	4.40	10.30			1.5	
St. John's												
Windsor-Parkers Br.	0.60	0.60	7.80	3.30		0.60					6.7	5
Windsor-Middle Rocky Br.		4.80	10.20	9.70		2.20			0.50		4.8	
Rennies River	15.60	7.80	2.10	10.40	7.80	3.10			1.00	2.60	2.1	0.5
Virginia River	6.40	4.10	8.70	16.30	0.60	1.20				1.20	2.3	1.2
Waterford River	1.60		0.00	21.90	8.90	3.60	1.00			0.50	0.5	7.8
North Avalon												
Salmon Cove			3.10	2.60	1.00	4.60	5.60				11.7	5.1
Chapel Arm	1.00										14.8	
Port Rexton					5.70	2.80	1.10				2.8	
South Avalon												
Chance Cove		5.10	29.60	0.50	0.50						4.6	6.6
Salmonier			2.70	0.00	2.20		1.10	17.00		3.30	34.1	
S E Placentia River				2.10				18.60		0.50	29.3	

Population	Alleles	Ssa410UOS continued										
		276	278	282	286	294	298	302	304	308	324	328
Scotland												
Loch Leven			5.40	4.10	4.10	1.40			1.40			
Howietoun					8.80		2.90					
St. John's												
Windsor-Parkers Br.			8.30		7.80			2.80			0.60	
Windsor-Middle Rocky Br.			8.60	2.20	2.20						1.10	
Rennies River				1.00	3.60	0.50	1.60	5.70	0.50			
Virginia River			2.30	0.60	4.10	0.60		0.00	0.60	0.60		
Waterford River			3.60	2.10	8.30	1.00		6.80				0.5
North Avalon												
Salmon Cove			6.10	2.60	5.10		1.00				1.50	1
Chapel Arm					1.50		5.60			0.50	1.50	3.1
Port Rexton				0.60	10.80		0.60				6.20	0.6
South Avalon												
Chance Cove		0.50	2.60	2.60		8.70			4.10			
Salmonier		1.60		2.70	8.80			11.00		0.50		
S E Placentia River		0.50		0.50	0.50							

Population	Alleles									Genes
		340	345	349	354	370	378	382	386	
Scotland										
Loch Leven				1.40				1.40		74
Howietoun										136
St. John's										
Windsor-Parkers Br.		1.70	1.10			2.80	0.60	0.60	3.30	180
Windsor-Middle Rocky Br.		2.70			0.50	2.70	0.50	0.50	4.30	186
Rennies River										192
Virginia River			0.60							172
Waterford River					0.50					192
North Avalon										
Salmon Cove		0.50	0.50	0.50	0.50	3.10	2.00		0.50	196
Chapel Arm						1.50			0.50	196
Port Rexton				4.50	4.50	4.50	0.00		6.80	176
South Avalon										
Chance Cove		1.00				1.50	1.00			196
Salmonier		0.50								182
S E Placentia River										188

Appendix IV, Chapter 3: Exact tests (Bonferroni corrected) showing lack of significant differences between temporal samples at each site.

	Ssa 416	Cocl Lav-4	One 9uASC	CA0 48828	Ssa 85	One 102-a	One 102-b	Ssa 406UOS	CA0 54565	QUB Str2	QUB Str3	CA0 60177	Ssa 197	Ssa D71	SaSa TAP2A	Ssa 410UOS
Howietoun	0.010	0.299	0.407	0.007	0.008	0.051	0.496	0.053	0.291	0.028	0.027	0.210	0.012	**0.002**	0.009	**0.000**
Parkers	0.121	0.645	0.622	**0.002**	0.019	1.000	0.023	**0.000**	-	**0.001**	0.166	0.258	0.015	0.018	0.934	0.015
Middle Rocky	0.611	0.228	0.013	0.520	0.463	1.000	0.040	0.270	-	0.749	0.004	0.109	0.444	0.216	0.069	0.690
Rennies River	0.820	0.038	0.068	0.746	0.246	0.505	0.254	0.016	0.008	0.066	0.613	0.060	0.011	0.117	0.678	0.486
Virginia River	0.629	0.141	0.658	0.043	0.078	0.432	0.101	0.137	0.204	0.161	0.298	0.398	0.089	0.644	0.204	0.087
Waterford River	0.374	0.177	0.924	0.276	0.033	0.528	0.011	0.027	0.280	0.003	0.364	0.337	0.063	0.830	0.087	0.403
Salmon Cove	0.549	0.268	0.022	0.026	0.359	0.254	0.114	0.076	-	**0.001**	0.068	0.027	0.083	0.079	0.278	0.046
Chapel Arm	0.080	0.556	0.086	0.282	0.543	1.000	0.013	0.723	0.471	0.005	0.722	0.034	**0.000**	0.004	0.799	0.099
Port Rexton	-	0.543	0.144	0.004	0.264	-	0.003	0.163	0.047	**0.000**	0.151	0.044	0.465	0.026	0.028	0.011
Chance Cove	-	0.149	0.736	0.023	0.038	0.135	0.069	0.151	0.267	0.091	0.586	0.227	0.004	0.433	0.062	0.023
Salmonier	-	0.099	0.419	0.585	0.090	0.383	0.065	0.281	0.124	0.471	0.045	**0.000**	0.174	**0.000**	0.212	0.038
SE Placentia	-	0.181	0.030	0.027	0.005	1.000	0.140	0.535	0.175	0.131	0.536	0.134	**0.000**	**0.000**	0.411	0.093

Chapter 4:

Population genetics of *Salmo trutta* L., from the Currane catchment, SW Ireland and individual assignment of rod-caught specimen sea trout from Lough Currane

Looking down on Lough Currane from below Lough Namaroon

Abstract

This study examined the patterns and levels of population genetic structuring of brown trout *Salmo trutta* from the Currane catchment, south-western Ireland, and the spawning provenance of large mature sea trout (the anadromous form of *S. trutta*) sampled from Lough Currane, using a genetic stock identification (GSI) approach. It is the first study of its kind on sea trout in an Irish lacustrine system. The baseline collection to enable GSI consisted of 0+ brown trout collected from all eight rivers draining into Lough Currane (373 fish). These were analysed for a panel of 16 neutral microsatellite loci, which were also applied to specimen sea trout (n=154). The latter were of average age of 6.5 years, and were rod caught in Lough Currane over a fifteen year period. ("Specimen" sea trout, as defined by the Irish Specimen Fish Committee, are those sea trout caught by rod and line which exceed 2.721kg or 6lbs in weight.) Population analysis of the juveniles, using the Bayesian STRUCTURE algorithm, showed evidence for two major groupings within the catchment, broadly demarked by geography, with the Cummeragh sub-catchment and Capal sub-catchment clustering separately. Further analysis, employing a hierarchical framework, showed significant structuring present between each river utilised in the baseline survey. Individual assignment analysis of sea trout showed that 90% of assignments were allocated to the Cummeragh sub-catchment (including the Finglas River). The Cummeragh River, which runs between the middle and lower lakes, was the largest contributor to the specimen sea trout in our sample, at 51%. The Finglas River, which flows into the inlet river to Lough Currane to the sea and which is outside the lake catchment proper, contributed 24% of the specimen fish caught in the lake and the Comavoher River contributed 15%. The Capal sub-catchment was the origin of only 10% of the specimen sea trout. These results reported here are relevant for identifying important evolutionary significant units of biodiversity, for the understanding of sea trout population dynamics and for the future management of this and other sea trout producing catchments.

4.1 Introduction

Brown trout, *Salmo trutta,* are known to show a wide range of life history strategies, including: freshwater resident (river trout), freshwater migratory (both to and within lakes, often referred to as lake trout), estuary migrants (slob trout), and anadromous forms (sea trout). In Ireland, the most commonly found life history for brown trout are those that migrate between spawning areas in rivers and feeding areas in downstream areas of rivers or in lakes (Ferguson, 2006). *S. trutta* are recognised as forming highly genetically structured populations, sometimes within the same catchments (Ferguson, 1989, Duguid, 2006). Highly structured populations are also common within large river systems in Ireland (M. O'Grady, Inland Fisheries Ireland, unpublished data) e.g. the Suir and Boyne rivers, which are large predominantly fluvial drainages in southern and eastern Ireland respectively. Reasons for this relate to their high level of natal homing and the different life-history strategies that can be utilised by different populations in the same area (Ferguson, 1989, 2004). The high level of genetic differentiation is likely a function of a combination of factors such as restricted gene flow and genetic drift and is also probably a product of adaptation to historical and contemporary environments.

Diversity in freshwater forms of brown trout has been studied in many lake environments. Differences, mostly between "ferox" trout and a lake resident morph have been reported in several European lakes (Ferguson, 1989, Ryman *et al.,* 1978, Duguid *et al.,* 2006, Massa-Gallucci, 2010). The population diversity of brown trout resident in lacustrine habitats is further discussed in Chapter One. Most of the structuring reported in previous studies relates to freshwater resident forms of brown trout, rather than differences between anadromous and freshwater resident forms. Anadromy in brown trout is attributed to a combination of genetic and environmental factors (discussed in Chapter 1). Genetic studies examining differences between sea trout and freshwater types cohabiting in freshwater have generally found no significant genetic differences, implying random mating and suggesting that interbreeding occurs between the two forms (Hindar *et al.,* 1991, Cross *et al.,* 1992, Pettersson *et al.,* 2001),. The lack of differentiation at the molecular marker level (neutral markers) does not necessarily indicate a lack of a genetic basis for

anadromy, as such studies do not take into account adaptive genetic traits (Ferguson, 2006).

Figure 1: Pre-spawning brown trout and sea trout. Images courtesy of Inland Fisheries Ireland, http://www.fisheriesireland.ie/fish-species.html

Sea trout are known to grow faster at sea than freshwater resident trout of the same age. This increased growth rate is clearly visible on the sea trout's scales and is one of the ways in which an anadromous life history can be identified. Anadromous forms of brown trout are also morphologically different to freshwater resident brown trout (Jonsson, 1985, Elliott and Chambers, 1996, Poole *et al.*, 2006, Figure 1). Sea trout populations along the west coast of Ireland experienced a serious population decline during the years 1989-1991, now thought to be at least partly related to increased environmental pressures caused by salmon farms along the migration routes of the fish, resulting in post-smolt stages of wild sea trout being infested by sea lice (*Lepeophtherius salmonis* and *Caligus elongatus*, Tully *et al.*, 1999, Poole *et al.*, 2006).

The Currane catchment, located in the south west of Ireland, escaped the worst of this decline, but a poor period of sea trout fishing was recorded on Lough Currane

for a period during the early 1990's. This was followed by a recovery to typical levels (Gargan *et al.*, 2006, Figure 2). Sea trout angling has a considerable importance in terms of tourism in many areas in Ireland, mainly along the western coast for fly-fishing, and more recently with an emphasis on estuarine and sea fishing. Sea trout populations in Ireland, both those affected by the decline of the early 1990's and those that were unaffected, are under increasing threat, both in terms of angling pressure and water quality issues in the freshwater environment (Anon, 1994, Poole *et al.*, 2006).

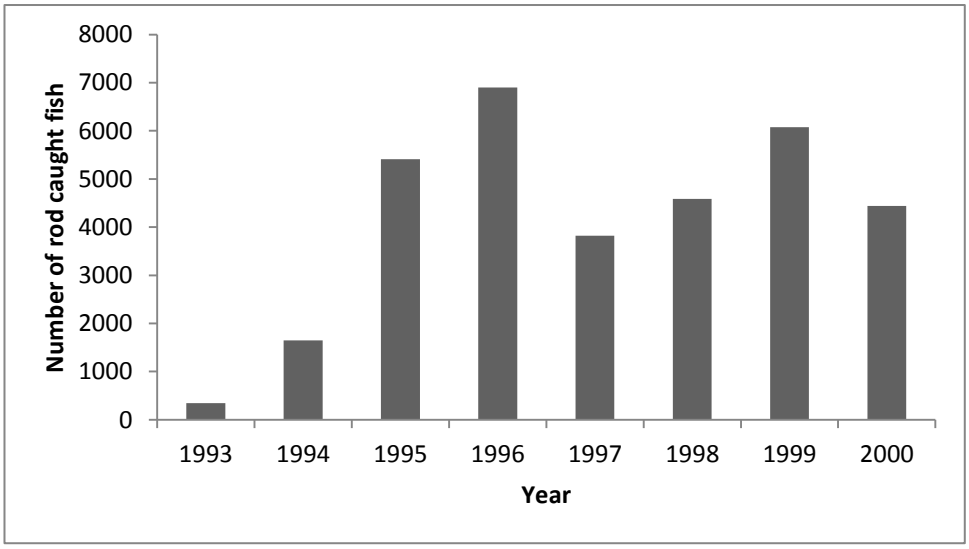


Figure 2: Sea trout rod catch in the Currane fishery for the years 1993-2000, adapted from Gargan *et al.* (2006)

The utilisation of genetic analysis in conservation and management of salmonid species has been discussed in Chapter 1. Methods such as Genetic Stock Identification (GSI) and its derivative, Individual Assignment (IA) analysis have been used extensively in the management of Pacific salmon species (genus *Oncorhynchus*) and more recently in Atlantic salmon, *Salmo salar* (Kalinowski, 2004, NGSI, 2008). These methods equally apply to brown trout management. The basic rationale of Genetic Stock Identification analysis (GSI) is to take genotypes of fish from populations that are thought to contribute to a mixed fishery (termed the baseline) and genotypes for the same loci from a mixed-stock fishery. If the population samples comprising the baseline are genetically distinct, using one of several currently available statistical methods, it is possible to identify the most likely origin of each fish belonging to the mixed fishery (i.e. individual assignment).

GSI and IA are found to be most accurate when: 1) most populations contributing to the mixture fishery are included in the baseline, 2) there is an adequate sample size, 3) when there is a high level of genetic differentiation between populations and 4) when a large number of loci are genotyped (Kalinowski, 2004, Anderson, 2008).

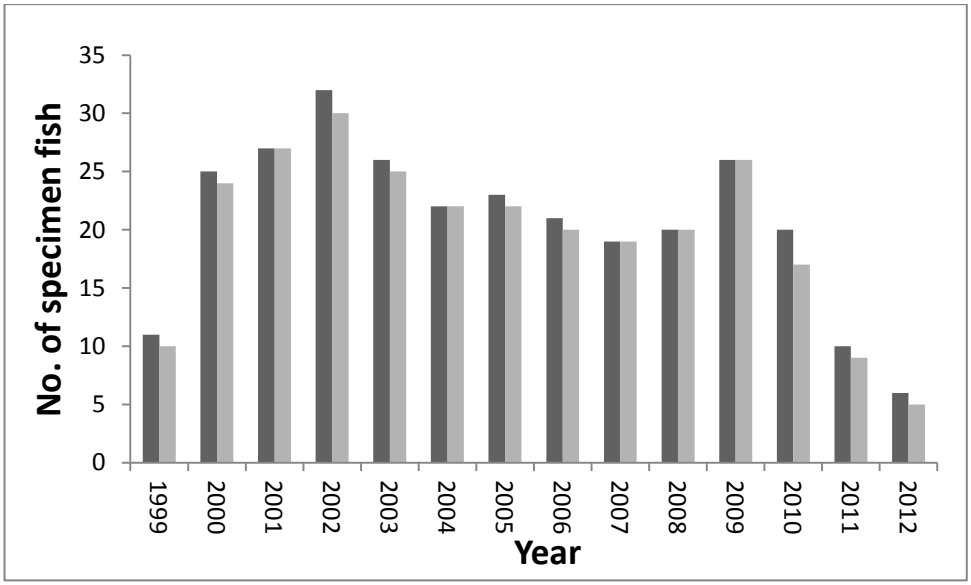


Figure 3: The total number (dark grey) of sea trout specimens received by the Irish Specimen fish Committee from Irish waters and the number from the Currane catchment (light grey).

The study site being examined here, the Currane catchment, is notable for its large, long-lived sea trout (Fahy, 1985) and currently contributes over 90% of the specimen sized sea trout submitted to the Irish Specimen Fish Committee in the years 1999-2012 (Figure 3). The system is known to have a high frequency of the *LDH-C1*100* allele, which is otherwise rare in sea trout populations but has been associated with large, long-lived ferox trout (Hamilton *et al.,* 1989, Duguid *et al.,* 2006). Sea trout in the Currane catchment are thought to be long-lived repeat spawners and although they have not been extensively studied, they have been historically recognised as particularly large and fast growing fish (Nall, 1930). The longevity of those fish recorded as specimen sea trout (average age of six to seven years), recorded from scale reading, contributes to their greater size at capture. An additional and important aspect is that they spawn multiple times.

In terms of management, an understanding of the location of spawning areas helps aid conservation efforts within the catchment. While brown trout are not protected

under the EU Habitats Directive, unlike Atlantic salmon, this species shows such variation across Ireland that more needs to be done to protect habitat and ensure the survival of different ecotypes.

4.1.1 Main Hypotheses of the Chapter-

The main hypothesis of Chapter Four was that the long-lived large sea trout found in Lough Currane could be assigned back to a river or region of origin within the Currane catchment.

This was based on the underlying hypothesis that population structuring would be observed between different rivers and sub-catchments of the Currane system. While GSI and IA analysis has been carried out previously on Atlantic salmon and resident brown trout populations in Ireland, no work has been done up to now on sea trout intra-catchment population variation. Thus, it was hoped that this study could add to our understanding of this topic.

While all main spawning areas within the Currane catchment were sampled as part of this investigation, nothing was known as to which areas in particular (or indeed if there are any such area), which produces the long-lived, large sea trout that make up the specimen fish sample studied here: If this study was to confirm that certain areas are responsible for the majority of the production of the valuable (in terms of angling revenue) specimen sized sea trout, this information could then be used to advise on future management and conservation.

4.2 Materials and Methods

4.2.1 Site Description

The Currane catchment is located in the south-west corner of Ireland in County Kerry (51°49’39 N, 10°07’25 W, Figure 4). It is within the Killarney National Park, Macgillycuddy's Reeks and Caragh River Catchment Special Area of Conservation (Site code 000365), with a total catchment area of 13,500ha. The Currane catchment is separated from the Inny catchment on the west by a continuous ridge. It is also separated from the neighbouring Caragh catchment to the north and the Sneem catchment to the east (O’Sullivan, 2012). The Geological Map of Ireland classifies the parent material of the soils in the catchment as Old Red Sandstone (Geological Map of Ireland, 1972). The catchment is underlain by upper Devonian sandstone with Lough Currane being underlain by the St. Finian sandstone formation (Geological Map of Ireland, 1972). The Environmental Protection Agency Corine Land Cover Map lists the bedrock of the Currane catchment as sandstone, shale till (originating in the Lower Palaeozoic) and peat. The subsoils are described as undifferentiated Aeolian sediments, some sandstone/shale till of Cambrian/Precambrian origin, blanket bog and undifferentiated alluvium gravel (EPA, 2006, O’Sullivan, 2012). There are five landcover types listed for the Currane catchment: pastures, peat bogs, natural grasslands, moors and heath lands and coniferous forests (EPA, 2006). The main agricultural activity in the catchment is drystock farming of both sheep and cattle. Turf cutting for domestic use is widely practiced within the catchment. In excess of 50% of the catchment comprises of Atlantic upland blanket bogs typical of western Ireland (EPA, 2006).

The nearest Met Eireann weather monitoring station is the Valentia Observatory (approx. 10 km from the catchment). 30-Year Average values show that the mean daily air temperature at the station is 10.4ºC, ranging from an average low of 4.2º C in January and February to an average high of 18º C in August. The average annual rainfall is 1430 mm, and the average daily duration of sunshine is 3.39 hours. Winds are predominantly from the south and west, with a mean yearly wind speed of 10.9 knots (Met Eireann, 2011).

The Currane catchment can be divided into two main sub-catchments, the Cummeragh sub-catchment to the north-east of Lough Currane, and the Capal sub-catchment, located to the south-west of the main lake. The Cummeragh River proper begins as an outfall of Lough Derriana, 116m above sea level, which drains into Lough Currane, at 4 metres above sea level. Loughs Adoolig and Tooreenbog are located above Lough Derriana and drain into this larger lake, and it also receives water from the outflows of two further lakes, Coomavannia (5.7 ha) and Nellinane (2.4 ha). Lough Derriana has an area of approx. 238ha. The Cummeragh is approximately 8.5km in length between Loughs Derriana and Currane, with two main tributaries, the Owengariff and the Owengarrihy (O'Sullivan, 2012). The Capal River flows for approx. 4km from source into Iskagahiny Lough (7ha), before continuing for another kilometre into Lough Currane. Along this stretch, it is joined by its tributary, the Cloughvoola River. Iskagahiny Lough also received inflow from Halliseys River, along with several smaller rivers. Lough Currane itself is an oligotrophic lake 1047 hectares in size that enters the sea through a small tidal opening (O'Sullivan, 2012). Flowing into the small, unnamed outflow river that connects Lough Currane to the sea is the Finglas River, which begins as the outflow of Coomoanig Lough, 5km upstream, in the south-western portion of the catchment.
The lakes of the Currane catchment are classified as acid oligotrophic. EPA assessments of the catchments over a twenty year period rate the water bodies as either good/high or high quality throughout the catchment (EPA, 2006).

The Currane catchment supports Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*), both resident and anadromous. A specimen sea trout in Irish waters is that which is heavier than 2.721 kg (or the traditional imperial measure of 6lb), with the record fish recorded at 7.428kg from the Shimna River in Co. Down in 1983 (ISFC, 2013). For 2012, out of six specimen sea trout recorded in Ireland, five were caught in Lough Currane. Overall for the last 13 years, 276 specimen Irish Sea trout were recorded as being caught in Lough Currane, out of a total of 288, i.e. 96% (ISFC, 2013, see Figure 3). Lough Currane and the rest of the Currane catchment support a salmon and sea trout fishery for a portion of the year, which provides tourism revenue to the nearby town of Waterville.

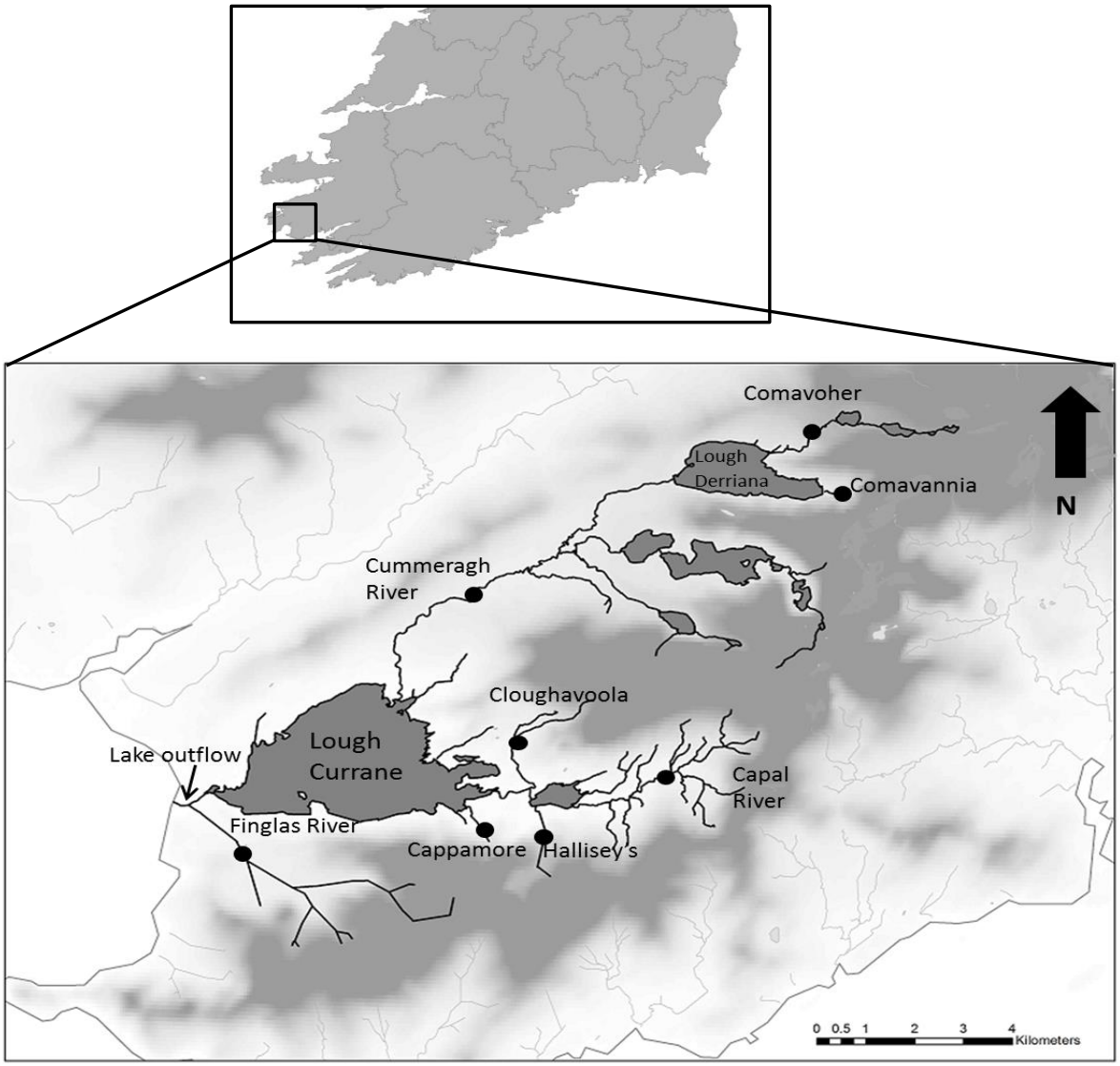


Figure 4: Location of rivers and streams sampled in the Currane catchment, Co. Kerry. Rivers are denoted by black lines, with lakes marked in grey and outlined in black. Sampling sites are marked by black dots, while gradient is indicated by grey scaling. White indicates land at lower than 100m elevation, light grey indicates land at between 100-250 m elevation and dark grey indicates land at an elevation of 250m or greater.

4.2.2 Sample collection

Juvenile trout were collected from eight tributaries throughout the Currane catchment, to form the baseline sample. These were regarded as the main spawning areas for brown trout in the catchment, based on previous surveys. A total of 373 0+ trout across eight sites were collected during August and September 2010. A single pass electrofishing method was used by trained workers (Smith Root LR-24 electrofisher) to collect juvenile trout (*Salmo trutta*). A long stretch of river was

sampled to minimise the possibility of collecting all samples from a few families (family effect). A minimum of 50 fish was sampled by electrofishing at each site. Juvenile trout were euthanatized by overdosing in clove oil and tissue sample consisting of muscle and caudal fin taken. These were preserved in 98% ethanol in individually labelled Eppendorf 1.5ml vials. Samples were brought to the Population Genetics Laboratory, School of BEES, UCC, at the end of the sampling season in 2010. Table 1 shows the breakdown of successfully analysed tissue samples from each site, along with approximate latitude and longitude for each of the areas sampled.

Table 1: Site names and sample numbers, along with latitude and longitude, for 0+ trout from spawning streams sampled by electrofishing during August and September 2010 in the Currane catchment, Co. Kerry, Ireland.

Site name	N	Latitude	Longitude
Cloughvoola	48	51°49'58.25" N	10°04'38.80" W
Capal	47	51°49'32.92" N	10°02'19.56" W
Halliseys	48	51°49'10.50" N	10°04'06.70" W
Cappamore	48	51°49'09.81" N	10°05'19.90" W
Finglas	48	51°48'53.73" N	10°09'56.75" W
Cummeragh	48	51°52'37.41" N	10°03'45.06" W
Comavoher	45	51°54'01.29" N	10°00'26.38" W
Comavannia	41	51°53'15.83" N	9°58'52.42" W

Adult samples consisted of sea trout scales collected from large sea trout by Ireland's Specimen Fish Committee for the years 1997 to 2010 inclusive. These 300 samples were made up of varying numbers of trout provided across the sample years (Table 2).

Table 2: Sample numbers of specimen sea trout scales sampled from Lough Currane in each year.

Year	1997	1998	1999	2000	2001	2002	2003
No. Specimens	13	25	9	23	27	26	26
Year	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**
No. Specimens	19	22	20	20	20	26	24

Scales were stored in paper envelopes for ageing and species verification purposes. A subsample of five scales was taken from this archive for genetic analysis. All

specimen trout were collected by rod by anglers during sea trout fishing season on Lough Currane.

4.2.3 Molecular analysis

Genomic DNA was extracted from tissue and archival scale samples by using the Puregene DNA extraction procedure (Qiagen Ltd), a salting-out method which gives high molecular weight, archival quality DNA. Scale samples of poor quality were re-extracted using a Qiagen scale kit (Qiagen Ltd.). Quantification of extracted DNA was carried out by spectrophotometry, using a Nanodrop ND-1000, and quality was assessed by running a subsample from each group of 96 individuals on a 1.5% Agarose gel.

Table 3: Panel information showing loci used, primer sequences (with ABI-labelled primer) and original literature references. Adapted from Keenan *et al.,* (2013)

Locus	Forward Primer	Reverse primer	Reference
Panel One			
Ssa416	FAM-TGACCAACAACAAACGCACAT	gtttCCCACCCATTAACACAACTAT	Cairney *et al.* (2000)
One103	FAM-GGGTACCCACTGACGCTATG	gttTCTGGTACTTCCCTGATGC	Olsen *et al.* (2000)
SsaD48	FAM-GAGCCTGTTCAGAGAAATGAG	gtttCAGAGGTGTTGAGTCAGAGAAG	King *et al.* (2005)
Cocl-Lav-4	VIC-TGGTGTAATGGCTTTTCCTG	gtttGGGAGCAACATTGGACTCTC	Rogers *et al.* (2004)
CA048828	VIC-GAGGGCTTCCCATACAACAA	gtttGTTTAAGCGGTGAGTTGACGAGAG	Vasemagi *et al.* (2005a)
One9μ	NED-CTCTCTTTGGCTCGGGGAATGTT	gtttGCATGTTCTGACAGCCTACAGCT	Schribner *et al. (1996)*
Ssa85	NED-AGGTGGGTCCTCCAAGCTAC	gtttACCCGCTCCTCACTTAATC	O'Reilly *et al.* (1996)
One102-a	NED-GGGATTATTCTTACTTTGGCTGTT	gtttCCTGGTTGGGAATCACTGC	Olsen *et al.* (2000)
One102-b	NED-GGGATTATTCTTACTTTGGCTGTT	gtttCCTGGTTGGGAATCACTGC	Olsen *et al.* (2000)
Ssa406UoS	NED-ACCAACCTGCACATGTCTTCTATG	gtttGCTGCCGCCTGTTGTCTCTTT	Cairney *et al.* (2000)
CA054565	VIC-TCTGTGGTTCCCGATCTTTC	gtttCAACATTTGCCTAGCCCAGA	Vasemagi *et al.* (2005b)
CA053293	PET-TCTCATGGTGAGCAACAAACA	gtttACTCTGGGGCATTCATTCAG	Vasemagi *et al.* (2005a)
Str2QUB	PET-CTGGGGTCCACAGCCTATAA	gtttGAGCTACAACCTGATCCACCA	Keenan *et al.* (2013)
One108	VIC-GTCATACTACTCATTCCACATTA	gtttACACAGTCACCTCAGTCTATTC	Olsen *et al.* (2000)
Panel Two			
Str3QUB	FAM-CTGACCGCTGCACACTAA	gtttGGCTCTAATCGACTGGCAGA	Keenan *et al.* (2013)
CA060177	VIC-CGCTTCCTGGACAAAAATTA	gtttGAGCACACCCATTCTCA	Vasemagi *et al.* (2005b)
Ssa197	VIC-GGGTTGAGTAGGGAGGCTTG	gttTGGCAGGGATTTGACATAAC	O'Reilly *et al.* (1996)
MHCI	PET-AGGAAGGTGCTGAAGAGGAAC	gtttCAATTACCACAAGCCCGCTC	Grimholt *et al.* (2002)
SsaD71	NED-AACGTGAAACATAAATCGATGG	gtTTAAGAATGGGTTGCCTATGAG	King *et al.* (2005)
SaSaTAP2A	gtttGTCCTGATGTTGGCTCCCAGG	NED-GCGGGACACCGTCAGGGCAGT	Grimholt *et al.* (2002)
BG935488	gttTGACCCCACCAAGTTTTTCT	NED-AAACACAGTAAGCCCATCTATTG	Vasemagi *et al.* (2005b)
Ssa410UoS	gtttGGAAAATAATCAATGCTGCTGGTT	PET-CTACAATCTGGACTATCTTCTTCA	Cairney *et al.* (2000)

PCR amplifications and the resolving of alleles were carried out using the methods described in Section 3.2.4 of Chapter Three. Details of loci used are given in Table Three.

From the original panel of twenty two loci, six loci amplified poorly for this particular set of samples and were therefore unreliable when it came to scoring (*Ssa406Uos, One103, One108, SsaD8, SsaD71, and BG9335488*). *MHC 1* was not used for this part of the analysis as it is not regarded to be a neutral marker (de Eyto *et al.,* 2011). The locus *One102* was found to be co-amplifying using the primer set for the "a" locus and was therefore separated into *One102-a* and *One102-b*. The finalised dataset contained fifteen loci which were used for data analysis: *Ssa416UoS, Cocl-Lav-4, One9μASC, CAO48828, Ssa85, One102-a, One102-b, CAO54565, CAO53293, Str2QUB, Str3QUB, CAO60177, Ssa197, SaSaTAP2A* and *Ssa410UoS*.

4.2.4 Statistical analysis

Data quality, consistency of PCR amplifications across loci, and inter and intra sample variation were assessed using the methods described in Section 3.2.5 of Chapter Three. Individual assignment analysis to identify the most likely origin of adult sea trout samples collected from Lough Currane to spawning river/region within the Currane catchment was carried out using both the programmes ONCOR, which uses a maximum likelihood approach (Anderson *et al*., 2008) and Geneclass 2, which uses a Bayesian approach (Piry *et al.*, 2004). GeneClass 2 was run using the Rannala and Mountain (1997) algorithm. Currently, there is no consensus as for best statistical approach for individual assignment. The distinct algorithms implemented in ONCOR and Geneclass fundamentally try to address the same question (i.e. individual assignments). It is thus considered reasonable to assume that instances were both methods agree are potentially more reliable. Results of assignments were compared, with any assignments that did not agree between the two approaches being discarded.

4.3 Results

4.3.1 Intra sample variation

Five hundred and twenty seven individual trout were analysed in this study. Of these, 373 represented baseline population samples collected from eight spawning streams, as parr and 147 adult specimen sea trout. Age and sex were not recorded for all specimen fish collected. However, those that were aged ranged between a minimum age of five and a maximum age of nine, averaging between six and seven years of age. Many were multiple spawners and, where sex was recorded, fish were predominantly female.

Table 4: Sample numbers of specimen sea trout scales sampled from Lough Currane in each year. The total number of specimen scale samples received, the number of successful DNA extractions and the percentage success rate are listed.

Year	No. Specimens	Successful Extractions	% Success
1997	13	6	46
1998	25	10	40
1999	9	7	78
2000	23	8	35
2001	27	10	37
2002	26	16	62
2003	26	7	27
2004	19	10	53
2005	22	9	41
2006	20	10	50
2007	20	11	55
2008	20	14	70
2009	26	22	85
2010	24	16	67

Problems with PCR amplification of DNA extracted from adult scale samples prevented further work on an additional 153 fish out of the initial sample of 300. Table 4 gives the number of original samples and the number of successfully amplified samples across each year sampled. Poor storage in some cases and visible mould are the most likely causes of the amplification problems A Qiagen scale kit was used on samples that failed to amplify using the Puregene salting out method. This second extraction method also failed in many cases, though usable DNA was

recovered from a further 35 fish. Many samples were stored in aluminium foil within their individual scale envelopes. This meant the scales did not dry out properly and mould was evident in many cases. Therefore, most of the resulting DNA was heavily degraded and thus unsuitable for PCR amplification. It was also found that, in general, older samples were not in poorer condition than the most recent (Table 4).

There was no evidence of linkage disequilibrium between the loci used in this study, nor was there evidence of natural selection acting on any loci. No problems with consistent genotyping errors were found with Micro-Checker. Testing for family effects, using COLONY software did not show any evidence of over-representation of family groups within any samples used in the analysis.

Table 5: Summary statistics for *S. trutta* samples screened for 16 microsatellite loci from the Currane catchment: ***N*** = number of individuals screened per sample: ***A*** (***T %***) = number of alleles (% observed in sample in relation to total observed among all samples).

Sample		Average	Total NA
Cummeragh	N	48	
	A (%T)	8.4 (69.2%)	135
Finglas	N	48	
	A (%T)	7.6 (62.1%)	121
Cloughvoola	N	87	
	A (%T)	6.9 (56.9%)	111
Cappamore	N	48	
	A (%T)	7.3 (60%)	117
Halliseys	N	48	
	A (%T)	7.3 (59.5%)	116
Capal	N	47	
	A (%T)	7.8 (63.6%)	124
Comavoher	N	42	
	A (%T)	7.4 (61%)	119
Comavannia	N	41	
	A (%T)	7.3 (60%)	117
Adult Sea Trout	N	147	
	A (%T)	9.4 (76.9%)	150

Summary statistics across all loci from all eight sampling sites and the adult samples are provided in Table 5 and Appendix I. The number of samples analysed at each locus are provided, along with the number of alleles and allele frequency for each locus. Allelic richness (A_R) and expected heterozygosity (H_E) were used to indicate within population genetic variability (Table 5, Appendix I). Observed (H_O) and expected heterozygosity (H_E) are presented, and significance values of HWE were corrected using a Bonferroni correction (0.05/16=0.003). No consistent deviations

from HWE (across samples) after a standard Bonferroni correction were found for any loci (Appendix I).

4.3.2 Inter sample variation

Allele frequencies are given in Appendix II. There were some differences visible in the allele frequency data for the streams sampled, indicating some possible level of structuring between rivers sampled, but no distinct patterns were obvious. Correlation assessment of distinct F_{ST} estimators (Figure 5) indicated G'_{ST} provided a good fit to the data, so this method was selected.

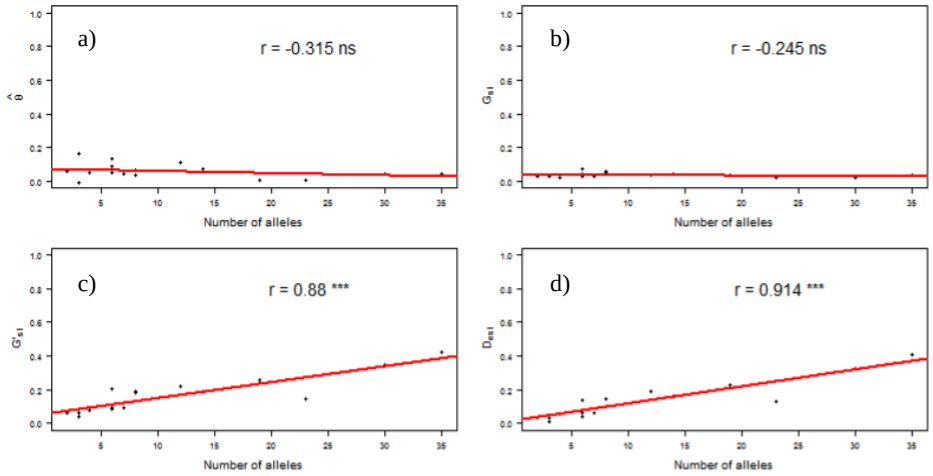


Figure 5: Correlation assessment of locus estimators a) F_{ST} (Weir and Cockerham, 1984), b) G_{ST} (Nei and Chester, 1983), c) G'_{ST} (Hedrick, 2005) and d) D_{est}, (D_{Jost} unbiased estimator, Jost, 2008), with locus polymorphism (total number of alleles), returned from the corPlot function in the DiveRsity software programme (Keenan *et al.,* 2013). Red lines represent the line of best fit and r values are Pearson product moment correlation coefficients. Both G'_{ST} and D_{est} provide a suitable fit to the data.

Population G'_{ST} values ranged from 0.0168 to 0.0408, with 95% confidence intervals all showing positive values, indicating statistically significant differences between sites, that is, that all sites were genetically distinct from each other (Appendix III). Table 6 shows geographic distances between baseline sampling sites within the Currane catchment. Looking at the values presented in Table 6 and Appendix III, there does seem to be a relationship between the level of genetic differentiation and geographic distance.

Table 6: Matrix of pairwise $G'_{ST,}$ (Hedrick, 2005) between sampling sites across all loci, on the bottom portion of the matrix, and distances (km) between sampling sites on the top portion.

	Cloughvoola	Capal	Halliseys	Cappamore	Finglas	Comeragh	Comavoher	Comavannia
Cloughvoola		6	3.4	4.8	13.2	15.9	20.3	21.2
Capal	0.0208		4.1	6	14.4	17.1	21.5	22.4
Halliseys	0.0193	0.0246		3.4	11.8	14.5	18.9	19.8
Cappamore	0.0316	0.0326	0.0336		9.7	12.5	16.9	17.8
Finglas	0.0227	0.0277	0.0172	0.0291		20.8	25.2	26.1
Cummeragh	0.021	0.0256	0.0188	0.0246	0.0168		4.4	5.3
Comavoher	0.0269	0.0309	0.0254	0.0258	0.022	0.0119		3.3
Comavannia	0.0408	0.0407	0.0336	0.0428	0.0305	0.027	0.0246	

An isolation by distance analysis (Mantel test) showed a strong positive relationship between geographical distance (km) and genetic differentiation (G'_{ST}) when looking at the Currane catchment sampling sites (r = 0.5074, p = 0.016, Figure 6). Allele frequencies and allele diversity are given in Appendix II. There were some patterns visible in the allele frequency data for the spawning streams looked at, indicating some level of structuring between rivers sampled, but no distinct patterns were obvious.

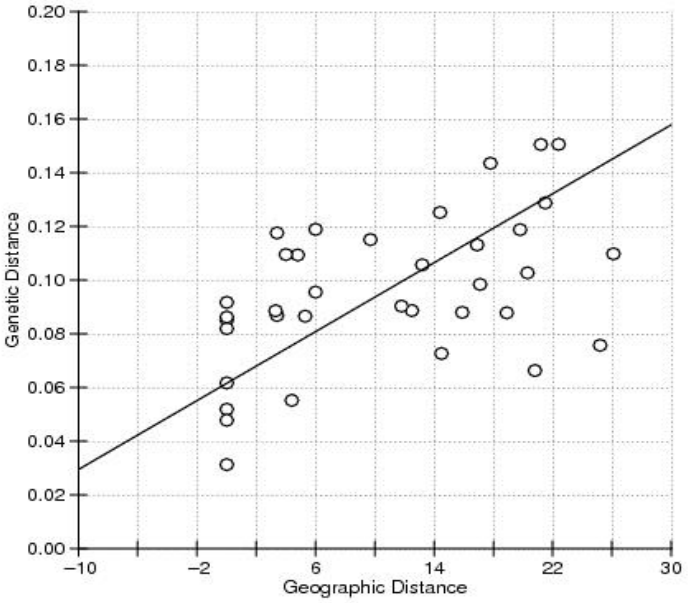


Figure 6: Isolation by distance analysis giving the relationship between geographic (km) and genetic distance (F_{ST}) for Currane catchment sampling sites. (r = 0.5074, p = 0.016).

Factorial component analysis (FCA) also showed evidence of clustering of samples (Figure 7) into geographical regions. The Finglas and Cummeragh sample

populations seemed to cluster together, as did the Comavannia and Comavoher. The spawning populations from the Capal sub-catchment (Capal, Halliseys and Cappamore) appeared to cluster together in the centre of the plot, with the exception of the Cloughvoola population (Figure 7).

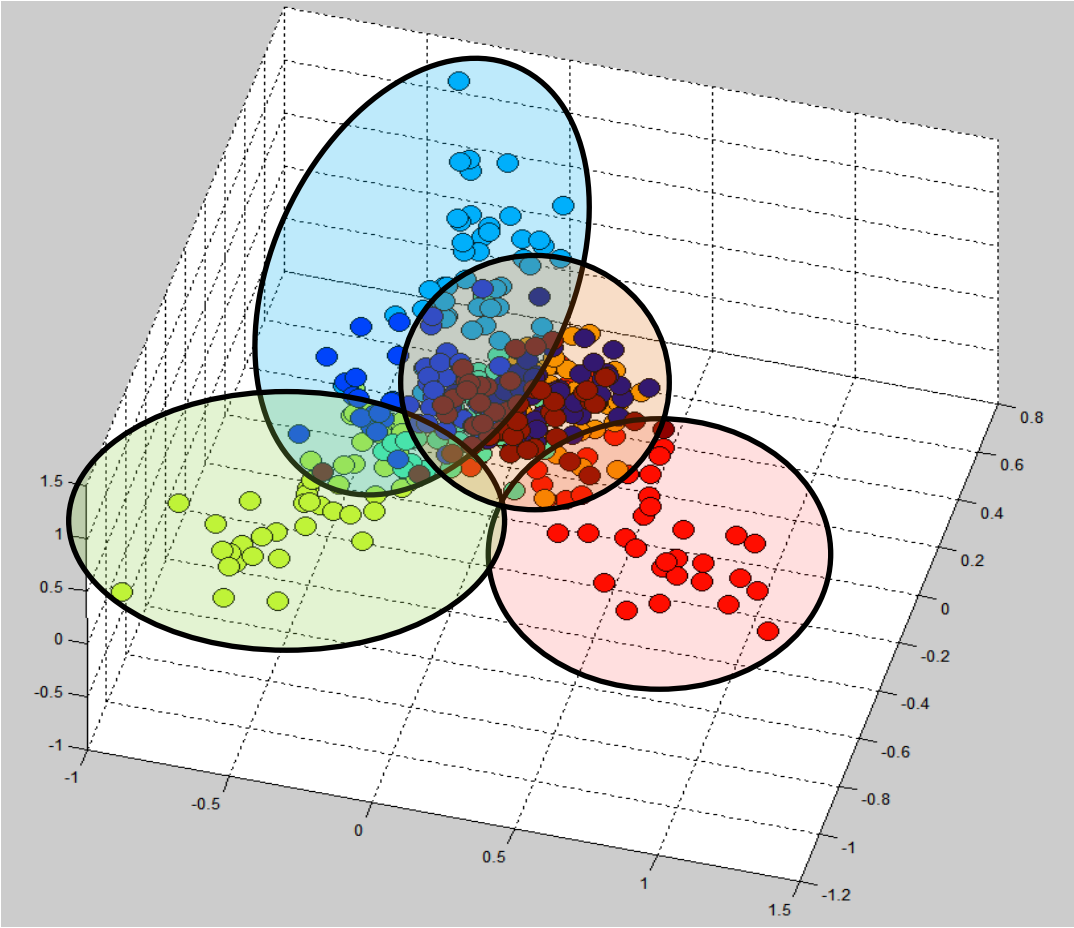


Figure 7: 3D Factorial component analysis of spawning stream brown trout samples from the Currane catchment. Blue grouping: Finglas (light blue), Cummeragh (mid blue), Comavoher (turquoise, also present in green grouping). Green grouping: Comavannia (green), Comavoher (turquoise, also present in blue grouping). Orange grouping: Capal (orange), Halliseys (brown), Cappamore (navy). Red grouping: Cloughvoola (red).

An unrooted neighbour-joining dendrogram, based on Nei's D_A, showed similar patterns of differentiation to those observed in previous analyses (Figure 8). The adult specimen fish sample clusters with Finglas, Comavannia, Comavoher and Cummeragh Rivers (Cummeragh catchment and Finglas River group of baseline populations). Cappamore, Halliseys, Cloughvoola and Capal samples (the Capal sub-catchment samples) seem to have less of a relation to the adult specimen fish sample, and cluster separately to the other samples, as seen in previous analyses.

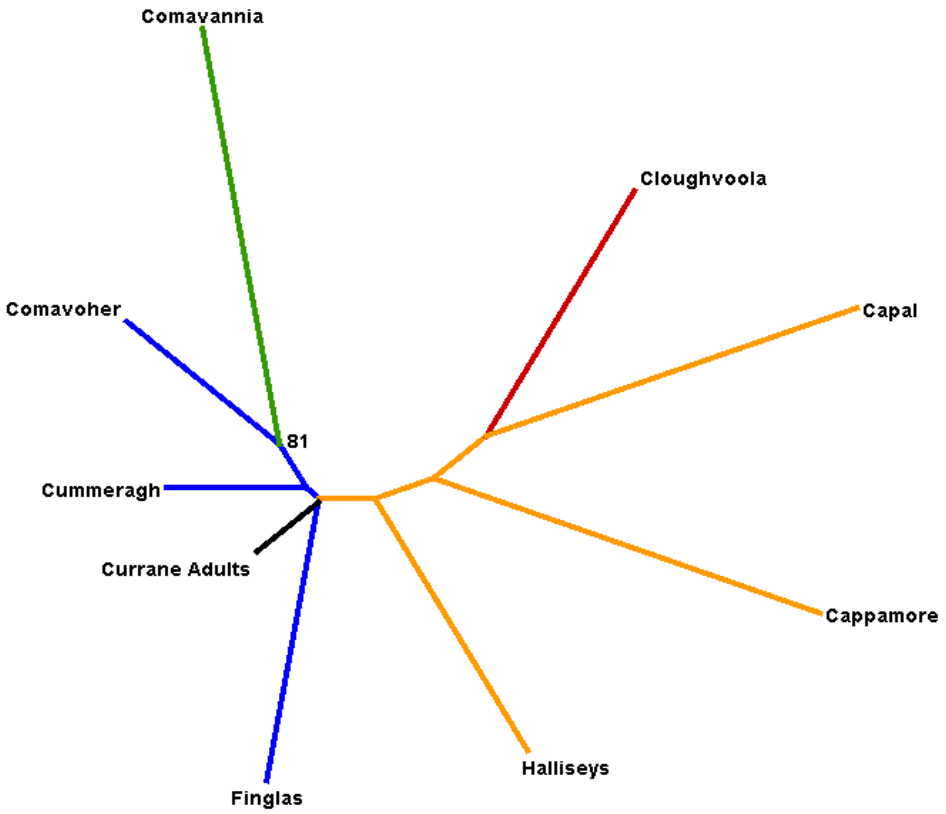


Figure 8: Unrooted neighbour- joining dendrogram based on Nei's D_A (1983) genetic distance illustrating relationship among baseline brown trout samples and adult sea trout samples, with bootstrap values higher than 85 indicated. Different colours represent major genetic groups as coded in FCA analysis.

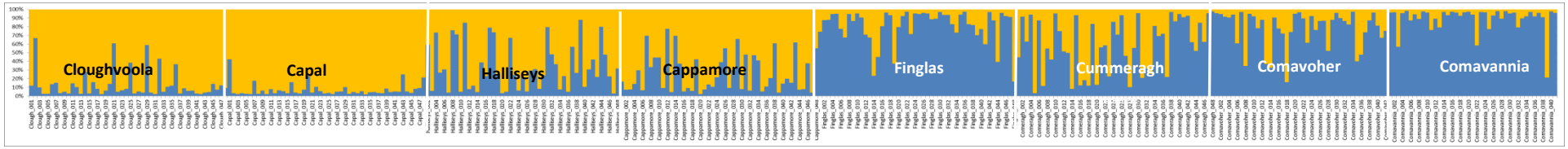
Results from the hierarchical STRUCTURE analysis of individuals show population structuring between regions within the catchment at the first hierarchical level (Figure 9). Thus, at the first level, the data is best explained by the presence of two genetic groupings (Table 7). Examining the sample membership of these two major groups (K=2, Figure 9a), indicates the existence of a split between the genetic structuring in the juvenile samples collected from sea trout spawning areas, with the Finglas, Comavannia, Comavoher and Cummeragh Rivers clustering separately from the Cappamore, Halliseys, Cloughvoola and Capal. These samples can be roughly divided into the Cummeragh sub-catchment and the Capal sub-catchment (Figure 4), with the exception of the sample from the Finglas River.

Table 7: Values of ΔK (Evanno *et al.*, 2005) giving all suggested values for K number of population groupings at two hierarchical levels. Peak values are highlighted in bold and were used as best estimates of K.

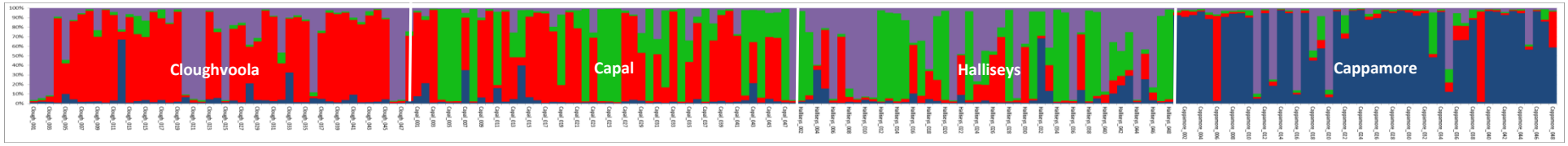
K value	Combined sites	Cummeragh sub-catchment and Finglas samples	Capal sub-catchment samples
	K=1 to K=15	K=1 to K=6	K=1 to K=6
1	-	-	-
2	**53.349***	5.235	4.707
3	6.329	**8.908***	2.835
4	4.752	4.057	**15.765***
5	0.114	0.334	0.180
6	0.795	-	-
7	0.032	-	-
8	3.55	-	-
9	3.117	-	-
10	1.22	-	-
11	0.166	-	-
12	0.507	-	-
13	0.073	-	-
14	0.671	-	-
15	-	-	-

Samples were divided and reanalysed based on suggested clusters provided by the first level of hierarchical analysis, giving bar plots at hierarchical level two (Figure 9 b and c). The bar plot of the second analysis, at the second hierarchical level (K=4, Table 6, Figure 9b), shows further structuring between the four baseline sample populations from the Capal sub-catchment. Looking at a bar plot of the STRUCTURE results for individuals in the Cummeragh sub-catchment, with the Finglas River (suggested K of three, Table 7, Figure 9c), the clusters generated by the software suggest that Comavannia is a distinct genetic cluster, while the Finglas, Cummeragh River and Comavoher populations appear to be quite similar.

a) Hierarchical Level One:



b) Hierarchical Level Two, Group One, Capal sub-catchment:



c) Hierarchical Level Two, Group Two, Cummeragh sub-catchment and Finglas River.

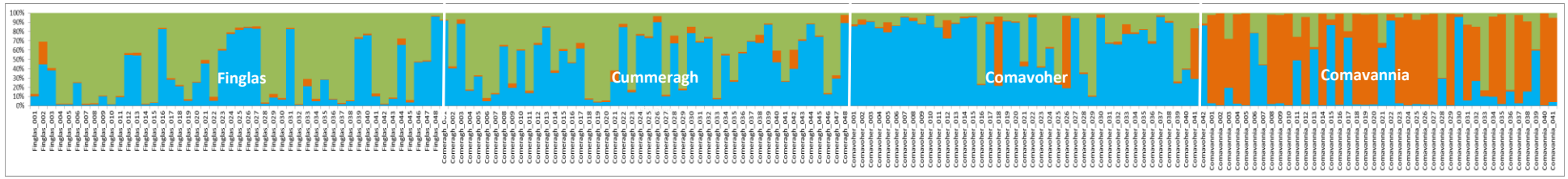


Figure 9 a) Bar plot of STRUCTURE population structuring simulations at K=2 for the baseline juvenile surveys carried out in the Currane catchment. Each bar represents an individual fish. Y-axis= proportion of each fish which can be attributed to each cluster, estimated from the colour of the bars. Samples are presented in geographical order, from the most easterly sample and following a clockwise direction around Lough Currane (see map in Figure 4). 9b) and 9c): Hierarchical Level Two bar plot of STRUCTURE population structuring simulations at K=4 (b) and K=3 (c) for the baseline juvenile surveys carried out in the Currane catchment, divided into a group based on Figure 9a. Colours in Figures 9b and c are independent of 9a and earlier analysis in Figures 7 and 8.

4.3.3 Individual assignment

Individual assignment analysis (Table 8) using the samples described above as the baseline was carried out on 154 individual adult sea trout collected over various years from Lough Currane using two different software programmes, ONCOR and Geneclass 2 and outputs were compared. Seventy seven per cent of assignments, or 118 individual fish, agreed between the two approaches (Appendix IV). Assignments that did not agree across the two software programmes used here, were deemed unreliable and so were removed from this analysis. Appendix IV gives ONCOR and Geneclass 2 likelihood scores for assignment to each baseline river sampled. Thirty six fish did not assign successfully using these criteria. Of the fish which did not meet the criteria, there was representation across almost all years sampled for specimen sea trout (Appendix IV).

Table 8: Individual assignment results, and percentage overall contributions collated from agreements between ONCOR and Geneclass assignment analysis on 154 adult sea trout from Lough Currane. Seventy seven per cent of assignments, or 118 individual fish, agreed between the two approaches, and represent an ONCOR percentage confidence in assignment of 80% or higher.

Sites	Contributions	Percentage
Cloughvoola	2	1.5%
Capal	2	1.5%
Halliseys	7	6%
Cappamore	1	1%
Finglas	28	24%
Cummeragh	60	51%
Comavoher	18	15%
Comavannia	0	0%
Total	*118*	*100%*

These results are shown in Table 8, both as counts and percentage representation. Interestingly, no adult specimen fish assigned back to the Comavannia, This river is quite distinct genetically from other rivers in the same geographical grouping (Figure 9c). The other three streams sampled as part of the Cummeragh sub-catchment and Finglas River grouping (Cummeragh, Finglas and Comavoher Rivers) together represent 90% of the assignment of specimen sea trout, with the Cummeragh River

being the main contributor at 51% representation. The other rivers sampled, which are all part of the Capal sub-catchment (Cappamore, Halliseys, Capal and Cloughvoola) together made up 10% of the contributions to the adult specimen fish sampled.

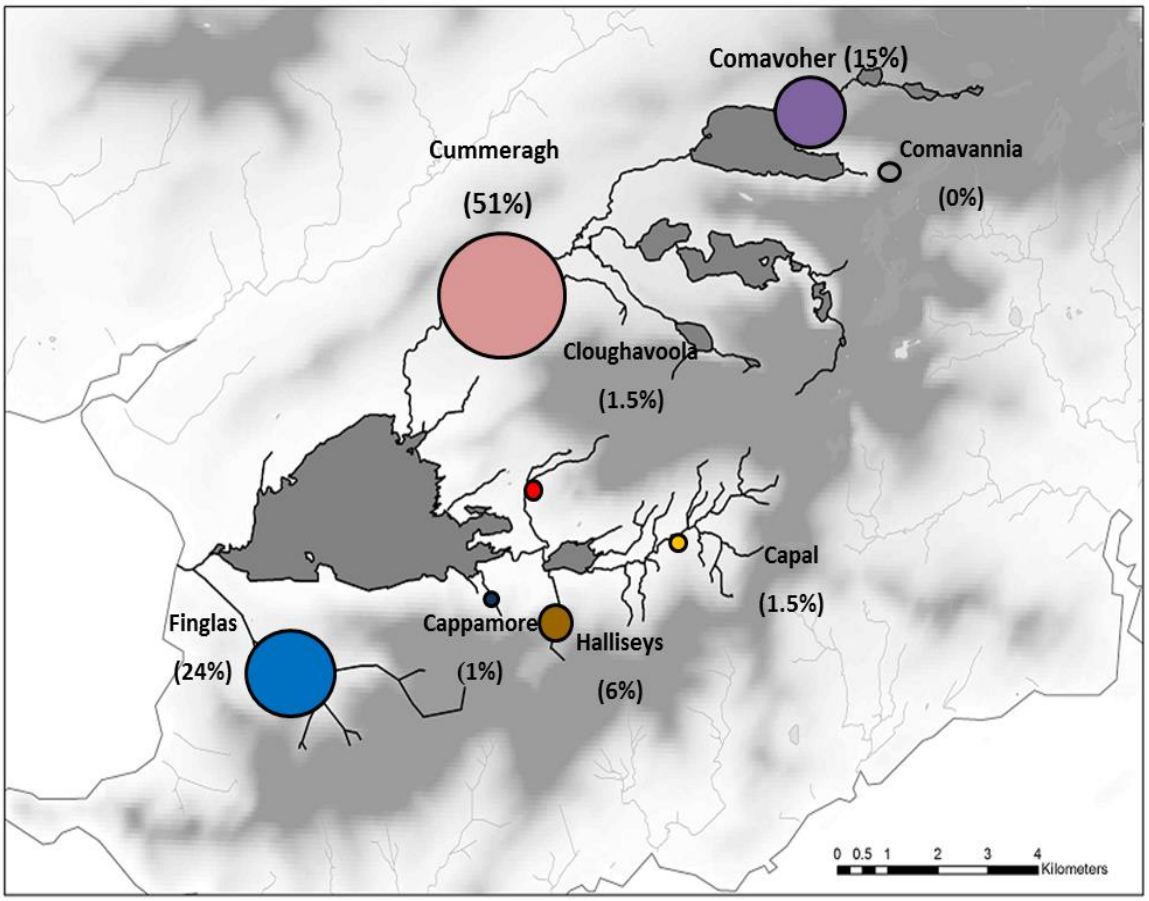


Figure 10: Genetic Stock Identification of specimen sea trout (i.e. relative contribution of each baseline river to specimen sea trout samples analysed, based on data in Table 8). The sizes of the pies in each case are indicative of the relative contribution of individual baseline-river. Numbers in parentheses represent actual contribution to the lake population sample in percentage.

Table 2, Appendix IV gives details of those fish that assigned to different rivers depending on the software used, and were not included in the analysis shown in Table 8 and Figure 10. A total of 36 individuals were not in agreement, with the majority of these (33 individuals) assigning to the Cummeragh River using the ONCOR analysis, but to a number of other rivers in the catchment when looking at the GeneClass output, which seemed split between the two sub-catchments (Table

9). These samples not in agreement also had assignment scores of between 50-80% in all cases, below the level accepted in this study as a robust assignment (80%).

Appendix V gives details of wetted area habitat available in the Currane catchment for each of the rivers sampled, and overall values for the catchment.

4.4 Discussion

Populations that are known to be geographically isolated (e.g. freshwater fish in different catchments) are more likely to show greater levels of genetic differentiation than populations with little or no isolation over a wide area (e.g. highly mobile marine fish species) (Avise, 1994). As a result, genetic markers are known to show increasing amounts of genetic variation when moving from marine to anadromous to resident freshwater species (Gyllensten *et al.,* 1984, Ward *et al.,* 1994, DeWoody and Avise, 2000). From this, we could expect that trout populations in river and lake systems which are dominated by anadromous phenotypes might be less differentiated than totally resident populations. A recent study on the brown trout of Lough Corrib in western Ireland, which is composed of mainly freshwater-resident trout with a small anadromous component, showed structuring, with separate demes assigning to different rivers within the system. The overall level of population sub-structuring among river samples was G'_{ST}= 0.095 for the Lough Corrib study (Prodöhl *et al.,* unpublished report). In the present study, an overall level of population sub-structuring among river samples of G'_{ST}= 0.027 was found, using mostly the same loci. This is consistent with the proposition of Gyllensten *et al.,* (1984), Ward *et al.,* (1995) and DeWoody and Avise, (2000). The genetic structuring of some trout populations in the Currane catchment could be, therefore, a product of the homogenising influence of anadromy in contrast to the differentiating effect of the lakes as promoter of genetic structure, where individual populations of trout with small numbers of breeders, isolated from each other by the lakes, can be strongly influenced by random genetic drift.

The Currane catchment is thought to have the only example of long-lived sea trout in Ireland (Fahy, 1994). Even populations of brown trout from neighbouring catchments, which also have an anadromous component, are known to be genetically distinct from brown trout in the Currane catchment and obtain neither the large size nor long life-span of the trout native to the Currane catchment (Gargan *et al.,* 2006). However, no work had previously been done on possible within-catchment population structuring of Currane catchment trout.

Results from this study show geographical structuring at two main hierarchical levels for baseline samples. At the highest level, the genetic data suggests two major population groupings within the catchment. These groupings cluster in terms of the Cummeragh sub-catchment with the Finglas River, and the Capal sub-catchment. At the second hierarchical level, our analysis showed evidence of some genetic differentiation between and among sites for all eight brown trout tributary populations sampled, which were then used as the baseline for genetic stock identification analysis.

The level of genetic differentiation varied between the two sub-catchment groups identified. Rivers sampled in the Capal sub-catchment appeared quite distinct, as has been seen previously for brown trout in Ireland (Ferguson, 2006). When looking at baseline sample structuring for the second sub-catchment group, there did not appear to be quite as high a level of differentiation, with the exception of the Comavannia sample. Differences between the Finglas, Cummeragh River and Comavoher samples were not as pronounced. The Comavannia sample stood out in this analysis because, while clustering with the Cummeragh sub-catchment grouping, it is also genetically somewhat distinct from them. High levels of brown trout genetic structuring have been found previously for freshwater resident trout in lacustrine environments (Ferguson, 1989, 2004, Duguid *et al.,* 2006 amongst others) and lakes have been recognised as promoters of genetic structuring in salmonids (Ramstad *et al.,* 2004, Dillane *et al.,* 2009). While these differences in the level of genetic structuring seen between sub-catchments may appear at first to reflect varying levels of natal homing within the wider catchment, it is as likely that these observed differences are a reflection of varying effective population sizes in the sub-catchments (Allendorf and Luikart, 2007). This study uses undifferentiated juvenile trout as its baseline sample, so we cannot assume that equal numbers of fish in each river sampled adopt an anadromous life history. If we take wetted area of the catchment as a proxy for trout productivity (Elliott, 1994), it can be estimated that the Cummeragh and Finglas sub-catchments would support larger populations of trout compared to the Capal sub-catchment (see Appendix V, wetted area). Therefore, these more productive areas would have larger populations and thus show less effect of genetic drift (Beebee and Rowe, 2004), a factor which can increase exponentially with decreasing population size. Also, it should be noted that

F_{ST} is not a reliable indicator of contemporary gene flow (and relatedly natal homing), as differences observed could reflect founder effects or other historical events, as discussed in Chapter Three.

The assignment of fish caught in the lake to the Finglas River is an interesting result in that it would suggest that some adult fish that enter the lake from the sea but prior to spawning exit the lake again in a seaward direction, to ascend a river which is located downstream of the lake. This would be a noteworthy trait variation associated with spawning behaviour in trout. While this behaviour has been noted previously for Lough Currane sea trout (Sean Clifford, pers comm.), based on observational data, the results presented here seem to back this up, as 24% of specimen sea trout tested, all of which were captured in Lough Currane, assign back to the Finglas River. This is a quite complex and possibly adaptive behaviour, which may have been selected to exploit the protection, afforded by the lake prior to spawning. As has been identified above, this trait fits into a continuum of adaptive behaviours and strategies associated with spawning in this species.

Anadromy in salmonids has been described as a threshold quantitative trait that is expressed due to a combination of environmental and genetic factors acting at a particular threshold level (Ferguson, 2006). Some work on the response of brown trout in the Burrishoole catchment, western Ireland, to catastrophic levels of marine mortality of post-smolts in the sea in the late 1980s and 1990s suggests marine migration in that catchment is mainly under genetic control (Poole *et al.*, 2006). It has been previously recognised that resident and anadromous brown trout can occur within the same population (Ferguson, 2006). However, due to population structuring found to occur in brown trout within catchments, sea trout from a particular catchment can be sourced from several distinct populations within the catchment (Ferguson, 2006). This concurs with what has been found here for the Currane catchment, where we show the first Irish example of sea trout assigning to distinct population groupings within one catchment, that is, adult fish that assign to the Capal sub-catchment and those that assign to the Cummeragh sub-catchment and Finglas River grouping (the latter of which forms the majority of sea trout examined in this study).

Individual assignment analysis showed that the Cummeragh River was the main contributor to the adult sea trout studied here (51%), with the Finglas and Comavoher Rivers having the next largest contribution. All these rivers form part of the Cummeragh sub-catchment and Finglas River grouping, with a fourth river, the Comavannia, showing no evidence of producing any specimen sea trout from those analysed. The Comavannia is a small river in terms of habitat (about 1% of overall area), so it possible that it contributes but our sample was not large enough to detect it. The Capal sub-catchment contributed a combined total of 10% of adult sea trout studied here.

If we look at the relative potential productivity of the rivers sampled for the baseline calculated only on wetted habitat area of streams in the immediate vicinity of the sample site (Appendix V), the relationship between habitat area and assignment results suggest habitat area may be the main factor determining contribution to specimen fish captured in Lough Currane, i.e. the largest rivers are the biggest contributors of specimen fish. The Cummeragh River was found here to be the largest contributor of the specimen sea trout at 51% of the fish caught, but is also largest river in the system with 55% of the habitat.

However, if the samples in the baseline are considered as been representative of a much wider geographical entity, for example, the wetted area of the entire Capal sub-catchment, which represents 30% of the system rather than 13%, then it would appear that these streams are considerably under-represented. They should be producing at least 30% of the fish based on habitat area, but appear to be only producing 10% in this study. Similarly, it would appear that the Finglas River is over represented producing almost 24% of the specimens with only 15% of the habitat. The Cummeragh and Comavoher are similarly over-represented producing together 66% of the fish with just over 50% of the wetted habitat area. This over representation of trout assigning to some rivers and under-representation in other may be an artefact of sampling undifferentiated juvenile trout.

The sampling strategy undertaken here was to collect juveniles from the main spawning areas for sea trout in the catchment. These sites were all understood to have anadromous and resident trout present during spawning times (based on annual

observations, Sean Clifford, pers. comm). It is possible that not all contributing spawning areas were sampled in this study, leading to a bias in the sampling design. A number of adult fish sampled assigned to the baseline at low confidence levels and were thus considered less likely to originate from baseline streams. This could also indicate that they are either possibly migrants from another area, are not sufficiently genetically differentiated to assign to a specific river, or they are part of another brown trout spawning aggregation within the catchment which was not sampled. The levels of genetic differentiation recorded between those spawning populations sampled within the Currane catchment were considered sufficient for assignment analysis (Anderson *et al.*, 2008), and to the best of our knowledge (and the considerable local knowledge of the Inland Fisheries Ireland staff who assisted with fieldwork), the sampling undertaken covered the major spawning areas within the catchment. However, it is still possible that an extension of the samples in baseline database could be beneficial in strengthening the IA results.

Summary and Conclusions

1) The Currane catchment in SW Ireland shows population structuring between the brown trout present. This population structuring could be divided by sub-catchments, and was more marked in Capal sub-catchment, indicating a stronger level of natal homing than was observed in the other sub-catchment grouping.

2) Specimen sea trout were found to assign mainly to three of eight rivers sampled in the baseline survey, which formed one of the sub-catchment groupings.

3) We have established the existence of different populations within the Cummeragh catchment and successfully assigned specimen sea trout back to their river of origin within the catchment, which adds to the knowledge of sea trout biology from the Currane and is the first such study involving Irish sea trout.

4) The maintenance of existing diversity within the Currane catchment should be a conservation priority. In agreement with Avise (1994), this study has shown that an understanding genetic population structure is critical for effective management as it provides a basis for defining management units and can identify populations of unusual genetic composition.

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Appendix I, Chapter 4: Summary statistics

Table 5: Summary statistics for *S. trutta* samples screened for 16 microsatellite loci from the Currane catchment: ***N*** = number of individuals screened per sample: ***A*** (***T%***)= number of alleles (% observed in sample in relation to total observed among all samples), ***Ho*** = observed heterozygosity, ***He*** = expected heterozygosity (Nei, 1987), ***A*r** = Allelic richness following the rarefaction method (Petit *et al.*, 1998), **HWE** = *P* values of exact tests for non-conformance to Hardy-Weinberg Average values per locus.

Sample		Ssa416	Cocl_Lav_4	One9uASC	CA048828	Ssa85	One102_a	One102_b	CA054565	CA053293	ppStr2	ppStr3	CA060177	Ssa197	MHCI	SaSaTAP2A	Ssa410UOS	Avg.	Total NA
Comeragh	N	48	48	48	48	48	48	48	48	48	48	48	48	48	48	48	48	48.0	
	A	3	4	4	26	4	2	14	2	5	15	3	11	5	9	8	20	8.4	135
	At%	100	57.1	66.7	66.7	100	100	73.7	66.7	62.5	57.7	42.9	78.6	71.4	75	100	66.7	69.2	135
	A_R	3.0	4.0	4.0	24.7	4.0	2.0	13.6	2.0	5.0	14.2	3.0	10.7	5.0	8.8	7.8	19.2	-	
	H_O	0.521	0.625	0.708	0.958	0.646	0.479	0.917	0.104	0.729	0.917	0.438	0.729	0.771	0.750	0.729	0.979	0.7	
	H_E	0.496	0.588	0.685	0.922	0.721	0.503	0.905	0.137	0.755	0.863	0.446	0.782	0.686	0.835	0.701	0.931	0.7	
	HWE	0.196	0.077	0.179	0.909	0.428	0.777	0.327	0.209	0.341	0.220	0.670	0.378	0.110	0.229	0.723	0.938		
Finglas	N	48	48	48	48	48	48	48	48	48	48	48	48	48	48	48	48	48.0	
	A	3	4	3	19	4	2	12	2	5	15	3	8	5	9	8	19	7.6	121
	At%	100	57.1	50	48.7	100	100	63.2	66.7	62.5	57.7	42.9	57.1	71.4	75	100	63.3	62.1	121
	A_R	3.0	4.0	3.0	18.2	4.0	2.0	11.6	2.0	4.8	14.3	2.8	7.8	4.8	8.6	7.8	18.5	-	
	H_O	0.458	0.750	0.479	0.958	0.604	0.479	0.958	0.042	0.792	0.875	0.438	0.688	0.625	0.917	0.667	0.938	0.667	
	H_E	0.466	0.663	0.581	0.917	0.711	0.495	0.830	0.154	0.759	0.885	0.513	0.762	0.636	0.824	0.695	0.926	0.676	
	HWE	0.674	0.475	0.115	0.014	0.024	1.000	0.080	0.001	0.374	0.831	0.376	0.231	0.586	0.550	0.035	0.005		

Sample		Ssa416	Cocl_Lav_4	One9uASC	CA048828	Ssa85	One102_a	One102_b	CA054565	CA053293	ppStr2	ppStr3	CA060177	Ssa197	MHCI	SaSaTAP2A	Ssa410UOS	Avg.	Total NA
Cappamore	N	48	48	48	48	48	48	48	48	48	48	48	48	48	48	48	48	48.0	
	A	3	4	4	20	4	2	11	3	5	8	4	11	5	9	8	16	7.3	117
	At%	100	57.1	66.7	51.3	100	100	57.9	100	62.5	30.8	57.1	78.6	71.4	75	100	53.3	60.0	117
	A_R	3.0	4.0	3.8	18.9	4.0	2.0	10.5	3.0	5.0	7.9	3.8	9.7	5.0	8.8	8.0	15.4	-	
	H_O	0.417	0.396	0.604	0.896	0.563	0.563	0.750	0.292	0.646	0.729	0.563	0.792	0.771	0.708	0.646	0.958	0.643	
	H_E	0.352	0.549	0.651	0.915	0.609	0.479	0.797	0.287	0.651	0.756	0.527	0.875	0.743	0.781	0.628	0.898	0.656	
	HWE	0.589	0.029	0.153	0.035	0.034	0.357	0.720	0.014	0.177	0.251	0.684	0.035	0.939	0.048	0.459	0.754		
Halliseys	N	48	48	48	48	48	48	48	48	48	47	48	48	47	47	48	46	47.7	
	A	3	6	4	20	4	2	10	2	8	10	4	8	5	8	6	16	7.3	116
	At%	100	85.7	66.7	51.3	100	100	52.6	66.7	100	38.5	57.1	57.1	71.4	66.7	75	53.3	59.5	116
	A_R	3.0	6.0	3.8	18.8	4.0	2.0	9.9	2.0	7.7	10.0	3.8	7.6	5.0	7.9	5.7	15.5	-	
	H_O	0.542	0.771	0.708	0.854	0.688	0.479	0.833	0.063	0.833	0.872	0.458	0.646	0.638	0.894	0.667	0.891	0.677	
	H_E	0.491	0.687	0.629	0.903	0.709	0.488	0.828	0.100	0.784	0.846	0.588	0.572	0.607	0.816	0.622	0.893	0.660	
	HWE	0.928	0.159	0.610	0.187	0.024	1.000	0.420	0.104	0.981	0.709	0.098	0.379	0.614	0.073	0.599	0.070		
Capal	N	45	47	47	47	47	47	47	47	46	47	47	47	47	47	47	47	46.8	
	A	3	4	4	18	4	2	12	3	7	13	4	11	4	8	6	21	7.8	124
	At%	100	57.1	66.7	46.2	100	100	63.2	100	87.5	50	57.1	78.6	57.1	66.7	75	70	63.6	124
	A_R	3.0	4.0	4.0	17.6	4.0	2.0	11.4	2.9	6.9	12.9	3.9	10.8	4.0	8.0	5.8	19.8	-	
	H_O	0.489	0.575	0.787	0.915	0.681	0.447	0.936	0.128	0.522	0.872	0.340	0.830	0.383	0.787	0.702	0.851	0.640	
	H_E	0.470	0.653	0.721	0.892	0.666	0.461	0.856	0.122	0.623	0.844	0.317	0.835	0.365	0.866	0.676	0.911	0.642	
	HWE	0.496	0.065	0.890	0.362	0.996	1.000	0.058	1.000	0.145	0.728	0.841	0.645	0.836	0.019	0.320	0.080		
Cloughvoola	N	48	48	48	48	48	48	48	48	48	48	48	48	48	48	48	48	87.4	
	A	3	4	4	16	3	2	11	2	5	11	3	7	5	10	8	17	6.9	111
	At%	100	57.1	66.7	41	75	100	57.9	66.7	62.5	42.3	42.9	50	71.4	83.3	100	56.7	56.9	111
	A_R	3.0	4.0	4.0	15.4	3.0	2.0	10.8	2.0	5.0	10.9	3.0	7.0	4.8	9.7	7.6	16.7	-	
	H_O	0.521	0.646	0.625	0.854	0.688	0.500	0.875	0.125	0.771	0.896	0.458	0.750	0.438	0.625	0.771	0.896	0.652	
	H_E	0.531	0.618	0.674	0.867	0.639	0.504	0.867	0.118	0.746	0.877	0.478	0.708	0.449	0.840	0.701	0.922	0.659	
	HWE	0.407	0.645	0.469	0.140	0.634	1.000	0.218	1.000	0.870	0.630	0.796	0.194	0.412	0.010	0.727	0.015		

Sample		Ssa416	Cocl_Lav_4	One9uASC	CA048828	Ssa85	One102_a	One102_b	CA054565	CA053293	ppStr2	ppStr3	CA060177	Ssa197	MHCI	SaSaTAP2A	Ssa410UOS	Avg.	Total NA
Comavoher	N	42	42	42	42	42	42	42	42	42	42	42	42	41	42	42	42	41.9	
	A	3	4	4	21	4	2	10	3	6	14	3	6	5	9	6	19	7.4	119
	At%	100	57.1	66.7	53.8	100	100	52.6	100	75	53.8	42.9	42.9	71.4	75	75	63.3	61.0	119
	A_R	3.0	4.0	4.0	20.8	4.0	2.0	10.0	3.0	6.0	13.7	3.0	6.0	5.0	8.9	6.0	18.9	-	
	H_O	0.738	0.548	0.619	0.952	0.691	0.476	0.905	0.357	0.714	0.976	0.286	0.714	0.585	0.595	0.786	0.881	0.676	
	H_E	0.637	0.664	0.651	0.942	0.694	0.501	0.855	0.359	0.729	0.857	0.364	0.754	0.739	0.779	0.722	0.945	0.699	
	HWE	0.075	0.369	0.897	0.078	0.439	0.765	0.387	1.000	0.578	0.441	0.085	0.516	0.044	0.128	0.841	0.022		
Comavannia	N	41	41	41	41	41	41	41	41	41	41	41	40	40	40	41	41	40.8	
	A	3	5	4	22	4	2	11	2	6	10	4	6	5	8	6	19	7.3	117
	At%	100	71.4	66.7	56.4	100	100	57.9	66.7	75	38.5	57.1	42.9	71.4	66.7	75	63.3	60.0	117
	A_R	3.0	5.0	4.0	21.8	4.0	2.0	11.0	2.0	6.0	10.0	4.0	6.0	5.0	8.0	6.0	18.9	-	
	H_O	0.561	0.732	0.537	0.902	0.878	0.244	0.951	0.073	0.683	0.805	0.342	0.825	0.675	0.825	0.610	0.878	0.658	
	H_E	0.604	0.625	0.524	0.917	0.728	0.318	0.869	0.071	0.685	0.816	0.377	0.775	0.659	0.767	0.620	0.929	0.643	
	HWE	0.425	0.611	0.707	0.172	0.257	0.150	0.644	1.000	0.487	0.463	0.272	0.775	0.811	0.357	0.310	0.160		
Adult Sea Trout	N	128	150	115	153	154	154	154	154	148	153	154	137	145	152	152	153	147.3	
	A	3	6	5	25	4	2	14	2	8	19	5	11	6	9	8	23	9.4	150
	At%	100	85.7	83.3	64.1	100	100	73.7	66.7	100	73.1	71.4	78.6	85.7	75	100	76.7	76.9	150
	A_R	3.0	5.3	3.7	21.3	4.0	2.0	12.9	2.0	6.8	14.1	3.9	9.4	5.2	8.2	7.6	19.6	-	
	H_O	0.516	0.587	0.678	0.863	0.760	0.546	0.903	0.143	0.750	0.797	0.474	0.737	0.676	0.704	0.618	0.928	0.667	
	H_E	0.529	0.643	0.649	0.931	0.723	0.501	0.889	0.133	0.764	0.848	0.487	0.756	0.706	0.824	0.661	0.937	0.686	
	HWE	0.440	0.046	0.205	<0.001	0.436	0.339	0.040	1.000	0.237	0.065	0.219	0.040	0.924	0.022	0.608	0.873		

Appendix II, Chapter 4: Allele frequencies

Allele frequencies and allele diversity for baseline stream samples and adult specimen sea trout sampled from the Cummeragh catchment, Co. Kerry

	Ssa416			
Population	**Alleles**			**Genes**
	122	**131**	**140**	
Cloughvoola	13.50	22.90	63.50	96
Capal	13.30	16.70	70.00	90
Halliseys	8.30	25.00	66.70	96
Cappamore	6.20	14.60	79.20	96
Finglas	9.40	20.80	69.80	96
Cummeragh	7.30	27.10	65.60	96
Comavoher	28.60	22.60	48.80	84
Comavannia	12.20	40.20	47.60	82
Adults				

	CoclLav4							
Population	**Alleles**							**Genes**
	153	**155**	**157**	**159**	**161**	**163**	**167**	
Cloughvoola		31.20	52.10	3.10	13.50			96
Capal		36.20	43.60	2.10	18.10			94
Halliseys	6.20	41.70	36.50	4.20	8.30	3.10		96
Cappamore	4.20	35.40	57.30	3.10				96
Finglas	20.80	26.00	47.90		5.20			96
Cummeragh	4.20	54.20	34.40		7.30			96
Comavoher	13.10	50.00	25.00		11.90			84
Comavannia	4.90	50.00	35.40		4.90		4.90	82
Adults								

	One9uASC					
Population	**Alleles**					**Genes**
	188	**200**	**202**	**204**	**214**	
Cloughvoola	2.10	24.00	34.40	39.60		96
Capal		18.10	39.40	27.70	14.90	94
Halliseys		15.60	46.90	36.50	1.00	96
Cappamore		43.80	35.40	19.80	1.00	96
Finglas		12.50	55.20	32.30		96
Cummeragh		18.80	34.40	40.60	6.20	96
Comavoher		20.20	45.20	33.30		84
Comavannia		4.90	61.00	32.90	1.20	82

				CA048828				
Population	**Alleles**							
	258	**260**	**262**	**264**	**266**	**268**	**270**	**274**
Cloughvoola	2.10	1.00		1.00	19.80		11.50	6.20
Capal	2.10			8.50	9.60		3.20	6.40
Halliseys	6.20		1.00	7.30	16.70	4.20	8.30	1.00
Cappamore	1.00	1.00	2.10	6.20	4.20	1.00	10.40	5.20
Finglas	6.20		1.00		9.40	2.10	4.20	11.50
Comeragh	3.10	2.10	3.10	3.10	21.90	1.00	1.00	7.30
Comavoher	6.00		4.80	3.60	6.00	1.20	6.00	7.10
Comavannia Adults				1.20	1.20		8.50	4.90

Population	**Alleles**							
	276	**278**	**279**	**280**	**282**	**284**	**286**	**287**
Cloughvoola	2.10	1.00				2.10	3.10	
Capal	3.20	27.70			5.30			
Halliseys		21.90		4.20		1.00	1.00	1.00
Cappamore	1.00	7.30			3.10		18.80	
Finglas	0.00	13.50		4.20	1.00	1.00	2.10	9.40
Cummeragh	2.10	12.50	4.20	2.10	2.10	3.10	5.20	1.00
Comavoher	1.20	9.50		6.00		6.00	1.20	3.60
Comavannia Adults		17.10	7.30	3.70	2.40	1.20	3.70	1.20

Population	**Alleles**							
	288	**289**	**290**	**292**	**294**	**296**	**298**	**300**
Cloughvoola		8.30		8.30				
Capal	3.20	2.10	7.40	4.30	4.30			1.10
Halliseys	2.10	5.20	5.20		3.10		3.10	
Cappamore				13.50	10.40	3.10	1.00	2.10
Finglas	16.70	1.00			5.20		2.10	
Cummeragh	5.20	1.00	2.10		3.10	3.10	3.10	1.00
Comavoher	3.60		2.40	1.20	4.80	4.80		
Comavannia Adults	2.40	1.20	3.70		1.20	2.40	8.50	18.30

Population	**Alleles**						**Genes**
	302	**304**	**306**	**314**	**318**	**320**	
Cloughvoola	2.10	26.00			2.10	3.10	96
Capal	3.20				5.30		94
Halliseys	1.00	5.20	1.00				96
Cappamore	1.00		4.20		3.10		96
Finglas		5.20					96
Cummeragh		4.20			1.00		96
Comavoher		15.50		3.60		2.40	84
Comavannia Adults	1.20	1.20			3.70	3.70	82

	Ssa85				
Population	**Alleles**				**Genes**
	110	**112**	**114**	**116**	
Cloughvoola	20.80	46.90		32.30	96
Capal	26.60	45.70	3.20	24.50	94
Halliseys	42.70	21.90	12.50	22.90	96
Cappamore	10.40	47.90	2.10	39.60	96
Finglas	35.40	31.20	7.30	26.00	96
Cummeragh	24.00	36.50	10.40	29.20	96
Comavoher	23.80	44.00	8.30	23.80	84
Comavannia Adults	28.00	25.60	11.00	35.40	82

	One102a		
Population	**Alleles**		**Genes**
	167	**170**	
Cloughvoola	47.90	52.10	96
Capal	64.90	35.10	94
Halliseys	59.40	40.60	96
Cappamore	61.50	38.50	96
Finglas	57.30	42.70	96
Cummeragh	46.90	53.10	96
Comavoher	54.80	45.20	84
Comavannia Adults	80.50	19.50	82

				One102b				
Population	**Alleles**							
	183	**195**	**199**	**203**	**207**	**211**	**215**	**223**
Cloughvoola		10.40	11.50	6.20	11.50	27.10	12.50	8.30
Capal	3.20	24.50	16.00	9.60	9.60	14.90	16.00	
Halliseys		20.80	31.20	2.10	3.10	11.50	6.20	9.40
Cappamore		11.50	32.30	1.00		4.20	3.10	26.00
Finglas		25.00	26.00	5.20	18.80	6.20	3.10	5.20
Cummeragh		14.60	12.50	8.30	16.70	7.30	10.40	6.20
Comavoher		26.20	20.20	10.70	11.90	7.10	9.50	
Comavannia Adults		13.40	6.10	12.20	24.40	3.70	2.40	3.70

Population	**Alleles**						**Genes**
	227	**231**	**235**	**239**	**243**	**267**	
Cloughvoola	1.00	4.20	5.20			2.10	96
Capal	1.10	1.10			1.10		94
Halliseys	10.40				3.10	2.10	96
Cappamore	14.60	3.10	1.00			2.10	96
Finglas	1.00	3.10		1.00	3.10		96
Cummeragh	9.40	4.20	1.00	2.10	3.10	3.10	96
Comavoher	4.80	4.80			1.20	3.60	84
Comavannia Adults	14.60	13.40		1.20	4.90		82

	CA054565			
Population	**Alleles**			**Genes**
	111	**113**	**115**	
Cloughvoola		93.80	6.20	96
Capal	1.10	93.60	5.30	94
Halliseys		94.80	5.20	96
Cappamore	2.10	83.30	14.60	96
Finglas		91.70	8.30	96
Cummeragh		92.70	7.30	96
Comavoher	1.20	77.40	21.40	84
Comavannia		96.30	3.70	82
Adults				

	CA053293						
Population	**Alleles**						**Genes**
	143	**151**	**155**	**157**	**159**	**163**	
Cloughvoola	2.10	32.30	26.00	12.50		27.10	96
Capal	4.30	8.70	19.60	5.40	3.30	57.60	92
Halliseys	14.60	18.80	30.20	4.20	3.10	27.10	96
Cappamore	4.20	7.30	53.10	13.50		21.90	96
Finglas	1.00	27.10	28.10	19.80		24.00	96
Cummeragh	6.20	10.40	26.00	22.90		34.40	96
Comavoher	7.10	16.70	45.20	11.90	2.40	16.70	84
Comavannia	2.40	17.10	50.00	15.90	1.20	13.40	82

	Ssa197					
Population	**Alleles**					**Genes**
	131	**135**	**139**	**143**	**147**	
Cloughvoola	11.50	72.90	6.20	8.30		96
Capal	11.70	78.70	3.20	6.40		94
Halliseys	9.60	58.50	10.60	19.10	2.10	94
Cappamore	19.80	37.50	14.60	25.00	3.10	96
Finglas	8.30	49.00	1.00	34.40	7.30	96
Cummeragh	19.80	26.00	5.20	45.80	3.10	96
Comavoher	25.60	29.30	9.80	32.90	2.40	82
Comavannia	16.30	48.70	5.00	28.70	1.30	80

	SaSaTAP2A								
Population	**Alleles**								**Genes**
	288	**316**	**318**	**322**	**324**	**326**	**328**	**330**	
Cloughvoola	50.00	1.00	7.30	1.00	13.50	10.40	14.60	2.10	96
Capal	51.10		1.10		3.20	19.10	13.80	11.70	94
Halliseys	56.20	1.00	10.40	1.00			9.40	21.90	96
Cappamore	58.30	3.10	3.10	2.10	10.40	14.60	3.10	5.20	96
Finglas	51.00	10.40	6.20	3.10	1.00	15.60	2.10	10.40	96
Cummeragh	51.00	12.50	6.20	1.00	2.10	11.50	8.30	7.30	96
Comavoher	45.20	17.90	7.10			20.20	4.80	4.80	84
Comavannia	30.50	53.70	2.40			3.70	4.90	4.90	82
Adults									

				Str2QUB				
Population	**Alleles**							
	213	**215**	**219**	**225**	**235**	**243**	**251**	**269**
Cloughvoola	7.30	2.10	4.20	12.50				
Capal	3.20	2.10	4.30	27.70			4.30	2.10
Halliseys	10.60		9.60	30.90				
Cappamore	2.10		3.10	33.30		5.20		
Finglas	5.20	3.10	15.60	12.50	5.20	4.20		
Cummeragh	1.00		11.50	12.50		5.20		2.10
Comavoher	1.20		7.10	15.50		1.20		1.20
Comavannia Adults	2.40		3.70	14.60				3.70
Population								
	279	**287**	**289**	**299**	**303**	**311**	**313**	**317**
Cloughvoola	12.50	7.30	3.10	22.90		11.50		2.10
Capal	5.30	2.10		25.50	5.30	8.50		
Halliseys	11.70	3.20	2.10	13.80		8.50		
Cappamore	2.10	14.60		33.30		6.20		
Finglas	9.40	6.20	1.00	24.00		4.20	1.00	6.20
Cummeragh	10.40	6.20	2.10	28.10		13.50		1.00
Comavoher	15.50	17.90	3.60	25.00		6.00	2.40	
Comavannia Adults	7.30	11.00		29.30		25.60		

Population				**Genes**
	333	**345**	**347**	
Cloughvoola		14.60		96
Capal		5.30	4.30	94
Halliseys	2.10	7.40		94
Cappamore				96
Finglas		1.00		96
Cummeragh	2.10	2.10	1.00	96
Comavoher	1.20	1.20	1.20	84
Comavannia Adults	1.20			82

		Str3QUB			
Population	**Alleles**				**Genes**
	129	**157**	**169**	**173**	
Cloughvoola	10.40	20.80	68.80		96
Capal	6.40	10.60	81.90	1.10	94
Halliseys	12.50	31.20	55.20		96
Cappamore	13.50	20.80	64.60		96
Finglas	1.00	45.80	53.10		96
Cummeragh	10.40	17.70	71.90		96
Comavoher	9.50	11.90	78.60		84
Comavannia Adults	6.10	11.00	78.00	4.90	82

				CA060177				
Population	**Alleles**							
	251	**259**	**263**	**267**	**268**	**271**	**272**	**275**
Cloughvoola				10.40		7.30		
Capal	1.10	6.40		25.50		2.10	6.40	4.30
Halliseys	1.00			18.80		6.20		1.00
Cappamore	1.00		12.50	20.80		14.60		1.00
Finglas	2.10		1.00	10.40		6.20		
Cummeragh	2.10	3.10		20.80	2.10	2.10		4.20
Comavoher				35.70		13.10		
Comavannia				15.00	10.00	18.80		
Adults								

Population						**Genes**
	276	**279**	**283**	**287**	**291**	
Cloughvoola	2.10	6.20	49.00	6.20	18.80	96
Capal	4.30	10.60	27.70	8.50	3.20	94
Halliseys		2.10	62.50	4.20	4.20	96
Cappamore	7.30	11.50	13.50	11.50	6.20	96
Finglas		24.00	38.50	15.60	2.10	96
Cummeragh		6.20	38.50	15.60	4.20	96
Comavoher		6.00	31.00	9.50	4.80	84
Comavannia		6.20	38.80	11.30		80
Adults						

				Ssa410UOS				
Population	**Alleles**							
	182	**200**	**204**	**208**	**212**	**213**	**216**	**217**
Cloughvoola				4.20				
Capal				1.10	1.10	1.10	9.60	1.10
Halliseys				1.10	2.20			
Cappamore					2.10		1.00	1.00
Finglas	4.20		3.10		6.20			
Cummeragh			1.00			2.10	2.10	
Comavoher		4.80						
Comavannia		3.70			8.50			
Adults								

Population								
	220	**224**	**228**	**232**	**236**	**237**	**240**	**242**
Cloughvoola	3.10	5.20	10.40	10.40	17.70		3.10	
Capal	14.90	1.10	4.30	20.20	3.20		5.30	
Halliseys			6.50	2.20	9.80		4.30	
Cappamore	3.10	4.20	10.40	13.50	20.80		13.50	
Finglas		1.00	3.10	5.20	10.40		9.40	
Cummeragh		4.20	12.50	12.50	10.40		4.20	
Comavoher		3.60	7.10	7.10	3.60		2.40	1.20
Comavannia			8.50	14.60	1.20	7.30	1.20	2.40
Adults								

Population								
	245	**249**	**253**	**257**	**262**	**266**	**270**	**274**
Cloughvoola	2.10	2.10	7.30	3.10			1.00	7.30
Capal	6.40	4.30	1.10	9.60	1.10		1.10	3.20
Halliseys		2.20	16.30	1.10		3.30	5.40	9.80
Cappamore	3.10		5.20	9.40		1.00	4.20	2.10
Finglas	18.80	5.20	3.10	5.20	1.00		7.30	4.20
Cummeragh	6.20	4.20	8.30	3.10		3.10	7.30	3.10
Comavoher	9.50	9.50	3.60	6.00	2.40	9.50	8.30	4.80
Comavannia	1.20	8.50	12.20	3.70	7.30	2.40	2.40	8.50
Adults								

Population						**Genes**		

	278	**282**	**286**	**290**	
Cloughvoola	8.30	5.20	2.10	7.30	96
Capal	4.30	3.20	3.20		94
Halliseys	21.70	10.90	1.10	2.20	92
Cappamore	5.20				96
Finglas	1.00	3.10	4.20	4.20	96
Cummeragh	10.40		2.10	1.00	96
Comavoher	2.40	4.80	2.40	7.10	84
Comavannia	1.20	3.70	1.20		82
Adults					

Appendix III, Chapter 4: Pairwise G'_{ST}

Matrix of pairwise $G'_{ST,}$ Hedrick, 2005) between sampling sites across all loci, on the bottom portion of the matrix, and 95% confidence intervals of pairwise F_{ST} on the top portion.

	Cloughvoola	Capal	Halliseys	Cappamore	Finglas	Comeragh	Comavoher	Comavannia
Cloughvoola		0.0147-0.0282	0.014-0.0258	0.0243-0.0401	0.0172-0.0298	0.0153-0.027	0.0192-0.0353	0.0299-0.0543
Capal	0.0208		0.0185-0.0319	0.0252-0.0414	0.0212-0.0358	0.0186-0.0334	0.0234-0.0395	0.031-0.0526
Halliseys	0.0193	0.0246		0.0258-0.0421	0.0119-0.0232	0.0129-0.026	0.018-0.0336	0.0243-0.0451
Cappamore	0.0316	0.0326	0.0336		0.0228-0.0366	0.0179-0.0326	0.0186-0.0336	0.0324-0.055
Finglas	0.0227	0.0277	0.0172	0.0291		0.0118-0.0229	0.0152-0.0306	0.0213-0.0417
Cummeragh	0.021	0.0256	0.0188	0.0246	0.0168		0.0067-0.0178	0.0172-0.0382
Comavoher	0.0269	0.0309	0.0254	0.0258	0.022	0.0119		0.0158-0.0364
Comavannia	0.0408	0.0407	0.0336	0.0428	0.0305	0.027	0.0246	

Appendix IV, Chapter 4: Assignment results

Table 1: ONCOR and Geneclass 2 assignments and likelihood scores for assignment,

Individual	Oncor	Probability	GeneClass	%	Agree
Currad97001	CurrB_Finglas	0.8471	CurrB_Finglas	83.231	YES
Currad97004	CurrA_Comeragh	0.9704	CurrA_Comerag	89.947	YES
Currad97007	CurrA_Comeragh	0.8929	CurrA_Comerag	66.81	YES
Currad97008	CurrG_Comavohe	0.9787	CurrG_Comavoh	96.706	YES
Currad97009	CurrB_Finglas	0.5728	CurrB_Finglas	53.463	YES
Currad97011	CurrB_Finglas	0.9491	CurrB_Finglas	64.495	YES
Currad98014	CurrA_Comeragh	0.9958	CurrA_Comerag	98.074	YES
Currad98015	CurrA_Comeragh	0.9104	CurrA_Comerag	74.554	YES
Currad98018	CurrA_Comeragh	0.7786	CurrC_Clough_	65.324	No
Currad98019	CurrA_Comeragh	0.6709	CurrG_Comavoh	66.952	No
Currad98022	CurrB_Finglas	0.7072	CurrB_Finglas	43.413	YES
Currad98024	CurrA_Comeragh	0.555	CurrE_Hallise	84.661	No
Currad98025	CurrC_Clough	0.5858	CurrC_Clough_	96.368	YES
Currad98031	CurrG_Comavohe	0.9658	CurrG_Comavoh	97.566	YES
Currad98032	CurrA_Comeragh	0.9968	CurrA_Comerag	98.532	YES
Currad98034	CurrG_Comavohe	0.523	CurrG_Comavoh	74.419	YES
Currad99039	CurrA_Comeragh	0.824	CurrD_Cappamo	77.453	No
Currad99040	CurrA_Comeragh	0.8151	CurrD_Cappamo	75.563	No
Currad99042	CurrG_Comavohe	0.8526	CurrG_Comavoh	95.152	YES
Currad99044	CurrB_Finglas	0.9962	CurrB_Finglas	99.869	YES
Currad99045	CurrA_Comeragh	0.9677	CurrA_Comerag	89.523	YES
Currad99046	CurrA_Comeragh	0.9993	CurrA_Comerag	99.716	YES
Currad99047	CurrG_Comavohe	0.713	CurrG_Comavoh	86.837	YES
Currad00049	CurrE_Hallisey	0.9951	CurrE_Hallise	98.57	YES
Currad00050	CurrA_Comeragh	0.9955	CurrA_Comerag	96.702	YES
Currad00051	CurrA_Comeragh	0.8476	CurrA_Comerag	47.697	YES
Currad00052	CurrB_Finglas	0.7984	CurrB_Finglas	60.931	YES
Currad00054	CurrB_Finglas	0.998	CurrB_Finglas	99.772	YES
Currad00055	CurrG_Comavohe	0.9563	CurrG_Comavoh	98.857	YES
Currad00058	CurrA_Comeragh	0.6196	CurrB_Finglas	55.12	No
Currad01073	CurrG_Comavohe	0.5049	CurrG_Comavoh	75.061	YES
Currad01074	CurrB_Finglas	0.9178	CurrB_Finglas	90.308	YES
Currad01075	CurrE_Hallisey	0.7185	CurrE_Hallise	94.897	YES
Currad01077	CurrB_Finglas	0.5189	CurrB_Finglas	36.194	YES
Currad01079	CurrA_Comeragh	0.9579	CurrA_Comerag	71.295	YES

Individual	Oncor1	Probability	GeneClass	%	Agree
Currad01081	CurrB_Finglas	0.5429	CurrB_Finglas	77.341	YES
Currad01082	CurrA_Comeragh	0.767	CurrB_Finglas	51.284	No
Currad01088	CurrG_Comavohe	0.9976	CurrG_Comavoh	99.903	YES
Currad01089	CurrE_Hallisey	0.5912	CurrE_Hallise	63.596	YES
Currad01090	CurrB_Finglas	0.5608	CurrD_Cappamo	45.956	No
Currad02099	CurrB_Finglas	0.6213	CurrB_Finglas	72.61	YES
Currad02100	CurrB_Finglas	0.6213	CurrB_Finglas	72.61	YES
Currad02101	CurrA_Comeragh	0.988	CurrA_Comerag	93.257	YES
Currad02102	CurrA_Comeragh	0.9689	CurrA_Comerag	88.053	YES
Currad02103	CurrG_Comavohe	0.5047	CurrG_Comavoh	79.983	YES
Currad02104	CurrA_Comeragh	0.9997	CurrA_Comerag	99.823	YES
Currad02107	CurrA_Comeragh	0.857	CurrD_Cappamo	69.703	No
Currad02108	CurrA_Comeragh	0.9976	CurrA_Comerag	98.857	YES
Currad02109	CurrA_Comeragh	0.9892	CurrA_Comerag	55.492	YES
Currad02110	CurrA_Comeragh	0.9105	CurrA_Comerag	40.826	YES
Currad02111	CurrA_Comeragh	0.8016	CurrF_Capal_0	39.723	No
Currad02112	CurrA_Comeragh	0.9235	CurrA_Comerag	74.399	YES
Currad02113	CurrA_Comeragh	0.9465	CurrA_Comerag	52.098	YES
Currad02114	CurrB_Finglas	0.792	CurrB_Finglas	90.33	YES
Currad02115	CurrG_Comavohe	0.6152	CurrG_Comavoh	84.544	YES
Currad02116	CurrA_Comeragh	0.8756	CurrA_Comerag	63.632	YES
Currad03124	CurrA_Comeragh	0.6214	CurrB_Finglas	52.399	No
Currad03132	CurrA_Comeragh	0.9808	CurrA_Comerag	91.367	YES
Currad03134	CurrA_Comeragh	0.9509	CurrA_Comerag	81.395	YES
Currad03136	CurrA_Comeragh	0.9961	CurrA_Comerag	98.356	YES
Currad03141	CurrA_Comeragh	0.9908	CurrA_Comerag	96.494	YES
Currad03143	CurrB_Finglas	0.561	CurrB_Finglas	49.958	YES
Currad03144	CurrA_Comeragh	0.9599	CurrA_Comerag	72.013	YES
Currad04150	CurrB_Finglas	0.5319	CurrB_Finglas	66.416	YES
Currad04152	CurrA_Comeragh	0.4575	CurrF_Capal_0	80.368	No
Currad04153	CurrA_Comeragh	0.7796	CurrE_Hallise	33.501	No
Currad04154	CurrA_Comeragh	0.9585	CurrA_Comerag	71.902	YES
Currad04156	CurrA_Comeragh	0.972	CurrD_Cappamo	66.103	No
Currad04157	CurrA_Comeragh	0.7658	CurrG_Comavoh	54.783	No
Currad04161	CurrD_Cappamor	0.7098	CurrD_Cappamo	86.285	YES
Currad04162	CurrB_Finglas	0.6425	CurrB_Finglas	86.115	YES
Currad04163	CurrA_Comeragh	0.8053	CurrA_Comerag	49.477	YES
Currad04166	CurrE_Hallisey	0.8523	CurrE_Hallise	98.286	YES
Currad05169	CurrB_Finglas	0.8541	CurrB_Finglas	86.918	YES
Currad05173	CurrA_Comeragh	0.7775	CurrD_Cappamo	71.842	No
Currad05175	CurrA_Comeragh	0.7894	CurrC_Clough_	72.195	No
Currad05176	CurrB_Finglas	0.7725	CurrB_Finglas	91.635	YES
Currad05177	CurrB_Finglas	0.982	CurrB_Finglas	95.189	YES
Currad05179	CurrA_Comeragh	0.6795	CurrG_Comavoh	63.893	No
Currad05180	CurrA_Comeragh	0.999	CurrA_Comerag	99.339	YES

Individual	Oncor1	Probability	GeneClass	%	Agree
Currad05183	CurrA_Comeragh	0.8686	CurrH_Comavan	63.177	No
Currad06190	CurrA_Comeragh	0.998	CurrA_Comerag	99.052	YES
Currad06191	CurrA_Comeragh	0.7572	CurrG_Comavoh	56.959	No
Currad06194	CurrG_Comavohe	0.8427	CurrG_Comavoh	89.712	YES
Currad06195	CurrB_Finglas	0.6033	CurrB_Finglas	73.626	YES
Currad06196	CurrA_Comeragh	0.7681	CurrA_Comerag	41.138	YES
Currad06198	CurrA_Comeragh	0.9476	CurrA_Comerag	79.698	YES
Currad06199	CurrA_Comeragh	0.9944	CurrA_Comerag	97.393	YES
Currad06200	CurrG_Comavohe	0.636	CurrG_Comavoh	72.105	YES
Currad06202	CurrA_Comeragh	0.6346	CurrG_Comavoh	59.581	No
Currad06203	CurrA_Comeragh	0.8843	CurrA_Comerag	61.244	YES
Currad07212	CurrA_Comeragh	0.7836	CurrG_Comavoh	33.003	No
Currad07214	CurrA_Comeragh	0.9949	CurrA_Comerag	92.974	YES
Currad07215	CurrA_Comeragh	0.8636	CurrA_Comerag	60.499	YES
Currad07216	CurrA_Comeragh	0.8636	CurrA_Comerag	60.499	YES
Currad07219	CurrA_Comeragh	0.4144	CurrF_Capal_0	61.669	No
Currad07221	CurrA_Comeragh	0.9964	CurrA_Comerag	90.076	YES
Currad07223	CurrA_Comeragh	0.9838	CurrA_Comerag	88.872	YES
Currad07225	CurrA_Comeragh	0.9303	CurrA_Comerag	72.481	YES
Currad07227	CurrA_Comeragh	0.9857	CurrA_Comerag	93.462	YES
Currad07229	CurrA_Comeragh	0.9989	CurrA_Comerag	99.522	YES
Currad07230	CurrA_Comeragh	1	CurrA_Comerag	99.976	YES
Currad08232	CurrB_Finglas	0.996	CurrB_Finglas	99.555	YES
Currad08233	CurrA_Comeragh	0.9902	CurrA_Comerag	80.36	YES
Currad08234	CurrG_Comavohe	0.981	CurrG_Comavoh	99.453	YES
Currad08236	CurrB_Finglas	0.6096	CurrB_Finglas	83.764	YES
Currad08237	CurrA_Comeragh	0.9183	CurrA_Comerag	72.986	YES
Currad08238	CurrA_Comeragh	0.9902	CurrA_Comerag	94.304	YES
Currad08239	CurrA_Comeragh	0.8773	CurrA_Comerag	62.807	YES
Currad08241	CurrE_Hallisey	0.9715	CurrE_Hallise	99.42	YES
Currad08242	CurrF_Capal	0.5554	CurrF_Capal_0	96.83	YES
Currad08243	CurrB_Finglas	0.8893	CurrB_Finglas	72.93	YES
Currad08244	CurrA_Comeragh	0.6388	CurrH_Comavan	94.952	No
Currad08246	CurrA_Comeragh	0.7148	CurrG_Comavoh	45.676	No
Currad08247	CurrA_Comeragh	0.7111	CurrE_Hallise	46.139	No
Currad08248	CurrA_Comeragh	0.8644	CurrA_Comerag	61.755	YES
Currad09252	CurrB_Finglas	0.5747	CurrF_Capal_0	80.466	No
Currad09253	CurrG_Comavohe	0.52	CurrG_Comavoh	71.438	YES
Currad09254	CurrA_Comeragh	0.6531	CurrG_Comavoh	68.51	No
Currad09255	CurrA_Comeragh	0.9782	CurrA_Comerag	84.52	YES
Currad09256	CurrA_Comeragh	0.8873	CurrA_Comerag	65.888	YES
Currad09257	CurrA_Comeragh	0.4937	CurrB_Finglas	52.174	No
Currad09258	CurrA_Comeragh	0.7498	CurrG_Comavoh	48.909	No
Currad09259	CurrA_Comeragh	0.7596	CurrG_Comavoh	54.508	No
Currad09260	CurrG_Comavohe	0.8779	CurrG_Comavoh	62.766	YES

Individual	Oncor1	Probability	GeneClass	%	Agree
Currad09261	CurrA_Comeragh	0.8684	CurrE_Hallise	41.532	No
Currad09262	CurrB_Finglas	0.7462	CurrB_Finglas	51.326	YES
Currad09263	CurrB_Finglas	0.5435	CurrB_Finglas	70.572	YES
Currad09264	CurrA_Comeragh	1	CurrA_Comerag	99.976	YES
Currad09265	CurrA_Comeragh	0.9957	CurrA_Comerag	82.725	YES
Currad09266	CurrA_Comeragh	0.7224	CurrB_Finglas	49.801	No
Currad09267	CurrA_Comeragh	0.9998	CurrA_Comerag	99.034	YES
Currad09268	CurrA_Comeragh	0.999	CurrA_Comerag	92.844	YES
Currad09269	CurrA_Comeragh	0.996	CurrA_Comerag	86.646	YES
Currad09270	CurrA_Comeragh	0.806	CurrE_Hallise	55.487	No
Currad09271	CurrB_Finglas	0.9959	CurrB_Finglas	98.831	YES
Currad09272	CurrB_Finglas	0.9485	CurrB_Finglas	68.416	YES
Currad09277	CurrA_Comeragh	0.5889	CurrE_Hallise	72.346	No
Currad10280	CurrE_Hallisey	0.993	CurrE_Hallise	95.391	YES
Currad10282	CurrB_Finglas	0.6497	CurrB_Finglas	81.364	YES
Currad10284	CurrG_Comavohe	0.7763	CurrG_Comavoh	85.349	YES
Currad10285	CurrA_Comeragh	0.9956	CurrA_Comerag	97.421	YES
Currad10286	CurrA_Comeragh	0.9971	CurrA_Comerag	98.201	YES
Currad10287	CurrF_Capal	0.7993	CurrF_Capal_0	82.211	YES
Currad10289	CurrG_Comavohe	0.5283	CurrG_Comavoh	72.33	YES
Currad10290	CurrA_Comeragh	0.8392	CurrA_Comerag	54.461	YES
Currad10291	CurrA_Comeragh	0.9055	CurrA_Comerag	69.87	YES
Currad10292	CurrB_Finglas	0.5331	CurrH_Comavan	67.177	No
Currad10295	CurrA_Comeragh	0.9903	CurrA_Comerag	78.924	YES
Currad10296	CurrG_Comavohe	0.9225	CurrG_Comavoh	94.103	YES
Currad10299	CurrA_Comeragh	0.9917	CurrA_Comerag	94.524	YES
Currad10300	CurrE_Hallisey	0.7371	CurrE_Hallise	96.104	YES
Currad10301	CurrA_Comeragh	0.9922	CurrA_Comerag	95.688	YES
Currad10302	CurrC_Cloug	0.7405	CurrC_Clough_	89.717	YES

Table 2: Individual assignments where there was not agreement between analysis methods used, data not accepted for assignment. Assignment values fell between 50-80% in all cases.

ONCOR		GeneClass	
Cummeragh River	33	Comavoher	10
		Finglas	5
		Comavannia	2
		Cummeragh sub-catchment	**17**
		Halliseys	6
		Cappamore	5
		Capal	3
		Cloughvoola	2
		Capal sub-catchment	**16**
Finglas River	3	Comavannia	1
		Cummeragh sub-catchment	**1**
		Cappamore	1
		Capal	1
		Capal sub-catchment	**2**

Appendix V, Chapter 4: Available wetted habitat

	Area (m^2)	Percentage contribution: Total area of rivers sampled, including all neighbouring streams	Percentage contribution: Total area of river habitat sampled in specific streams
Cummeragh (all including mountain lake streams)	147743	46.69	71.54
Cummeragh (up as far as Derriana Lough)	115595	36.53	55.97
Comavoher	11021	3.48	5.34
Comavannia	3413	1.08	1.65
Finglas	48464	15.31	23.47
Total Western streams sampled	**178493**	**56.40**	**86.43**
Total Western streams	**210641**	**66.56**	
Halliseys	6213	1.96	3.01
Cloughavoola	14553	4.60	7.05
Cappamore	2160	0.68	1.05
Capal	5109	1.61	2.47
Total Eastern streams sampled	**28035**	**8.86**	**13.57**
Total Eastern streams	**97401**	**30.78**	
Total catchment (excluding all mountain lake streams)	288171		
Total catchment	316457		
Total area of rivers sampled	206528		

Chapter 5:

General Discussion

This thesis has demonstrated new insights into the population structuring of salmonids and is also an example of the power of microsatellite markers, when used in a population genetics context, to demonstrate levels of genetic structuring. This chapter will demonstrate how the three separate investigations together contribute to new understandings of how salmonid populations behave between and within catchments.

Between catchment genetic population structuring in salmonid species has previously been believed to not impact greatly on fitness at small geographical scales (less than 50 km). Chapter Two, however, provided evidence to suggest that inter-river local adaptation in Atlantic salmon can occur at these scales, and is substantial in extent. The importance of this result is that it is contrary to the conclusions of Adkison (1995) and Meier *et al.* (2011), who, based on modelling studies, suggested that current knowledge suggested that adaptations, important for fitness, would be expected to occur at larger regional scales in salmonids. Something thought to be critical to interpreting the spatial scale of adaptation in a species, however, is that species' dispersal capability (Richardson *et al.,* 2014). Although salmon and trout are renowned for displaying natal homing, straying among rivers is known to occur (Ferguson, 2006) and genetic evidence suggests that limited dispersal among distant regions is not uncommon (Dionne *et al.,* 2008). It is possible therefore that a conflict exists in salmonids at the intra-regional scale between the diversifying effects of spatially variable selection and the homogenising effects of gene flow, meaning that local adaptation of populations cannot be either presumed or disregarded at this scale. Brown trout are thought to stray more frequently than Atlantic salmon (Ferguson, 2006, Chapter Three). Brown trout are also considered more successful invaders of new habitats (in a contemporary sense) than Atlantic salmon (Ayllon *et al.*, 2004, Launey *et al.*, 2010).

While the neighbouring populations of Atlantic salmon in Chapter Two showed significant differences in F_{ST} at neutral microsatellite markers, less differentiation was found between these two Atlantic salmon populations from different catchments than between some of the brown trout population groupings studied in the Cummeragh catchment in Chapter Four, although it should be noted that a different set of loci was examined for Atlantic salmon than for brown trout. Atlantic salmon, with its high proportion of anadromy within a population, is known to show lower levels of genetic differentiation when compared to brown trout population structuring (Ferguson, 1989, 2004), as this work shows.

Brown trout as introductions to Newfoundland showed no evidence that observed lowered genetic diversity in neutral markers at increasing geographic distance from the original site of invasion had impacted on the species' ability to establish in new habitats up to this point (Chapter Three). This could be related to the large adaptive potential of the brown trout as invaders, recently postulated for Newfoundland trout populations by Westley *et al.* (2013). Previous work have also shown that bottlenecks can reduce variability at neutral loci without effecting, or having only a small effect, on quantitative trait variation, the driving force behind adaptive variation (Dlugosch and Parker, 2008, Purcell *et al.*, 2012). The relative importance of the opposing forces of reduced genetic variation and adaptive evolution associated with dispersal is an important consideration in determining how readily salmonids will adapt to new environments and challenges, such as changing climate (Jonsson and Jonsson, 2009), recolonisation of rivers recovering from a history of industrial pollution (Ikediashi *et al.*, 2012) or the potential of introduced hatchery fish to establish populations in vacant habitats (Milner *et al.*, 2004).

Genetic population structuring at the within catchment level was also examined by this thesis. As discussed in Chapter Four, lakes are recognised as promoters of genetic structuring in salmonids (Ramstad *et al.*, 2004, Dillane *et al.*, 2009), with one of the suggested reasons being their limiting effects on within-river migration (Dillane *et al.*, 2009). Distinct populations as seen in the Currane catchment, in this work (Chapter Four) and also in the Rennies River catchment (Chapter Three) seem to show evidence of this effect. Populations that are known to be geographically isolated (e.g. salmonids in tributaries running into different parts of a lake) are more

likely to show greater levels of genetic differentiation than populations experiencing physical homogeneity over a wide area (e.g. highly mobile marine fish species) (Gyllensten, 1985, Avise, 1994). There was a substantial level of genetic structuring among brown trout populations in the Currane system with two major clusters discriminating populations in the Cummeragh and Capal sub-catchments. While Lough Currane would appear to have a major influence on the distribution and number of discrete populations observed, it is noteworthy that the level of differentiation is substantially less than has been found in other Irish lake systems, where resident trout rather than sea trout predominate (Fahy, 1985, Ferguson, 2006). This difference tells us something about the biology of sea trout and could be related to the number of migrants exchanged per generation (Ward *et al.,* 1994). Presumably sea trout populations within a system exchange more migrants than resident trout populations, since our results show less structuring in the populations in Cummeragh sub-catchment which were found to produce the largest proportion of sea trout. From this, we could expect that trout populations in river and lake systems which are predominately based on anadromous phenotypes might be less differentiated than totally, or partially resident populations. The genetic structuring of some of the trout populations in the Cummeragh catchment is likely therefore to be a product of the homogenising influence of anadromy in contrast to the differentiating effect of the lake as promoter of genetic structure, where individual populations of trout with small numbers of breeders, isolated from each other by the lake, will be strongly influenced by random genetic drift.

Atlantic salmon and brown trout are threatened throughout their native range, with problems including habitat destruction and fragmentation, fishing pressure, increased parasite and disease load from fish farming and invasive species (Ferguson, 2006, McGinnity *et al.,* 2009, Jonsson and Jonsson, 2009) Future climate change effects are also expected to place pressure on existing salmonid populations (Fealy *et al.*, 2012, Jonsson and Jonsson, 2009). This thesis has provided further evidence for the need to preserve genetic variability and diversity across both small and large geographic scales for both Atlantic salmon and brown trout in their native range. If the differences in survival found in Chapter Two are typical for Atlantic salmon, then supplemental stocking of populations, or continuous farmed escapes, are likely to have a negative long- term effect on effective population size and cause a loss of

genetic diversity (McGinnity *et al.*, 2009). Previous work has demonstrated that brown trout stocked into a virgin site are more likely to successfully survive than stocked Atlantic salmon (Launey *et al.*, 2010*)*. However, hatchery effects could reduce genetic diversity and therefore possibly reduce the success of some brown trout strains used for stocking. Local adaptation of native populations could also provide an advantage in terms of persistence, as suggested by the work of Hilborn *et al.*, (2003), McGinnity *et al.* (2009) and Schindler *et al.* (2010).

This research has provided new insights into the scale of local adaptation in salmon, the ecology of brown trout as a biological invader and the link between intra-river quantitative genetic variation and molecular genetic variation. While these chapters look at the existence and scale of genetic variation from different angles, it might be concluded that the overarching message from this thesis should be to highlight and promote the importance of maintaining genetic diversity in salmonid populations as vital for their long-term productivity and resilience and ultimately to their management as resource for socio-economic benefit.

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General Appendix I: Supplementary Information

Co-authorship of paper:

de Eyto, E., McGinnity, P., Huisman, J., Coughlan, J., Consuegra, S., Farrell, K., O'Toole, C., Tufto, J., Megens, H.-J., Jordan, W., Cross, T. and Stet, R. J. M. 2011. Varying disease-mediated selection at different life-history stages of Atlantic salmon in fresh water. *Evolutionary Applications*, **4,** 749-762. onlinelibrary.wiley.com/doi/10.1111/j.17524571.2011.00197.x/full

Talks Given:

- "Determining the scale of biologically important local adaptation in Atlantic salmon using a common garden experimental approach". Talk given at the International Symposium of Advances in the population ecology of stream salmonids. May 2010, Luarca, Spain.
- "Determining the scale of biologically important local adaptation in Atlantic salmon using a common garden experimental approach" Talk given at the Annual meeting of the American Fisheries Society, 2011. September 2011, Seattle, USA.
- Annual updates presented at the 2009 (Pitlochry, Scotland) and 2010 (Bordeaux, France) meetings of the European Network for Salmonid Postgraduate Researchers (NowPas)

Posters Presented: Both posters presented at the Beaufort Mid-Term Review, April 2012.

- Local adaptation in Atlantic salmon (*Salmo salar* L.): seeking empirical evidence within a common garden experimental framework (the Owenmore experiment)
- Using genetic markers to provide an insight into the ecology of sea trout (*Salmo trutta* L.) from Lough Currane, Ireland

Travel Grants Awarded:

- European Network for Salmonid Postgraduate Researchers (NowPas) travel costs to 2010 and 2011 annual meetings
- Ireland-Newfoundland Travel Grant, April 2010: €2,500 to facilitate 10 week visit to Memorial University, St. John's Newfoundland to carry out fieldwork and build ties with Newfoundland researchers (Dr's Ian Fleming and Peter Westley)
- Fisheries Society of the British Isles Travel Grant, August 2011: £1,000 to contribute towards costs of attending the American Fisheries Society Annual Conference, Seattle, September 2011
- School of Biology, Environmental and Earth Sciences, UCC travel grant, August 2011: €800 to contribute towards of attending the American Fisheries Society Annual Conference, Seattle, September 2011
- University of Montana, August 2011: awarding of flights from Seattle from Missoula and six weeks accommodation on campus at the University of Montana to facilitate visit to Prof. Fred Allendorf's laboratory, attending classes and undertaking training in laboratory techniques